# Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis


**A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE RULES AND REGULATIONS FOR THE MD PEDIATRICS DEGREE EXAMINATION OF THE TAMILNADU DR.M.G.R MEDICAL UNIVERSITY TO BE HELD IN MAY2018**

# CERTIFICATION

This is to certify that the dissertation titled **"Serum Calprotectin levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis "** is the bonafide original work done by Dr. Anish Sam George under my guidance during his academic term (2015-2018), in Pediatric Rheumatology under Pediatric Unit II at Christian Medical College, Vellore in partial fulfillment of the requirement for MD Pediatrics Degree examination of Tamil Nadu Dr.MGR Medical University, Chennai to be conducted in May 2018.

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# DECLARATION

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URKUND

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**Abstract:**

**Title:** SerumCalprotectin levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis

**Background:**Juvenile idiopathic arthritis (JIA) is the most common chronic rheumatic disorder of childhood and encompasses a complex group of disorders comprising several clinical entities with the common feature of arthritis. Calprotectin is a calcium- and zinc-binding protein that belongs to the S100 protein family and is released during the interaction of leucocytes with inflammatory activated endothelium at the sites of inflammation as occurs in JIA. We undertook this study to assess the usefulness of Calprotectin as a marker of disease activity in Indian children with JIA.

**Objectives:** To assess the usefulness of serum Calprotectin levels as a marker of disease activity in children with JIA.

**Materials and methods:** 121 children who fulfilled the International League of Associations For Rheumatology (ILAR) criteria for JIA were recruited into the study. Baseline demographic details were collected and Blood counts, ESR, CRP and Calprotectin levels were analyzed in all children after obtaining consent. Children were then divided into 2 groups based on disease activity as per Wallace criteria. Calprotectin levels were also analysed in 10 normal healthy children. Calprotectin levels weremeasured by using a “Human Calprotectin Kit” which works on the basis of sandwich-enzyme linked immune sorbent assay technology (ELISA).

**Results:** 121 children with JIA were recruited into the study, 63 had active disease and 58 had inactive disease. Systemic onset JIA constituted 42% of the study population and was the predominant disease subtype. Calprotectin levels were elevated in children with active disease compared to those with inactive disease. Mean Calprotectin value in active disease (3954ng/ml) was 2 fold higher than those with inactive disease (1899ng/ml) (p value <0.001)and 16 times higher than children who were normal healthy controls(mean of 233ng/ml). Area under curve for Calprotectin was 0.744 . For a cut off value of 1760 ng/ml, Calprotectin had a sensitivity of 77% and specificity of 61% for assessment of disease activity in JIA.

**Conclusion:** Serum Calprotectin levels was found to be a good marker of disease activity in children with JIA. However, further studies which involve serial monitoring of Calprotectin levels in a study population will provide additional information about accuracy of these markers.

***Keywords: Juvenile idiopathic arthritis, Calprotectin, disease activity.***

# Contents

# INTRODUCTION

Juvenile idiopathic arthritis (JIA) encompasses a complex group of disorders comprising several clinical entities with the common feature of arthritis. . It is not a single disease and encompasses different types of arthritis which has onset before the age of 16 years, symptoms last for more than 6 weeks and are of unknown cause. Each subtype of Juvenile Idiopathic Arthritis is characterized by distinct method of presentation, varied signs and symptoms, course and outcome. It is the most common chronic rheumatic disorder of childhood and is characterized by varied symptoms such as fever, joint pain, joint swelling, restriction of movements and can also involve other constitutional symptoms such as anorexia, weight loss and growth restriction.

Even though, clinical criteria for JIA is fairly well defined, appropriate laboratory parameters for diagnosis and assessment of disease activity is still lacking. Current lab parameters being used in JIA include erythrocyte sedimentation rate, C-reactive protein (CRP), antinuclear antibody, rheumatoid factor (RF) and cyclic citrullinated peptide (CCP) antibody.JIA is a disorder in which inflammation is at the core of the pathogenesis of the disease and hence various biomarkers which serve as markers of inflammation are an area of active research in this condition. A biomarker is any molecule which can be measured in accessible patient material such as blood or urine, is easily measurable and remains stable over time and gives valuable information regarding various aspects of disease such as disease activity, prognosis and response to treatment. Various biomarkers are currently under research in children with JIA and one such biomarker is serum

Calprotectin. Calprotectin is a granulocyte and monocyte complex of calcium- and zinc-binding proteins that belong to the S100 protein family and is released during cell activation and turnover. It is released during the interaction of leucocytes with inflammatory activated endothelium at the sites of inflammation as occurs in JIA. In recent years, few studies have reported the usefulness of Calprotectin as a marker of disease activity in children with JIA. But most of these studies incorporated only 1 or few subtypes of JIA. Studies from India which looked at Calprotectin levels in children with JIA are very few. Hence, we undertook this study to assess the usefulness of Calprotectin as a marker of disease activity in Indian children with JIA incorporating all the disease subtypes and also to compare Calprotectin with ESR and CRP which are currently the 2 main investigations used for assessment of disease activity.

# REVIEW OF LITERATURE

## JUVENILE IDIOPATHIC ARTHRITIS

Juvenile Idiopathic Arthritis (JIA) is a term used to describe a clinically heterogeneous group of arthritides of unknown cause. It is not a single disease and encompasses different types of arthritis which has onset before the age of 16 years, symptoms last for more than 6 weeks and are of unknown cause. (1) Each subtype of Juvenile Idiopathic Arthritis is characterized by distinct method of presentation, varied signs and symptoms, course and outcome. Previously, this condition was called Juvenile Chronic Arthritis or Juvenile Rheumatoid Arthritis (JRA). However, the currently accepted nomenclature internationally is Juvenile Idiopathic Arthritis (JIA).

### EPIDEMIOLOGY:

JIA is the most common chronic rheumatic disorder of childhood.(1) In developed countries, it has a yearly incidence of 2–20 cases per 100,000 population and a prevalence of 16–150 cases per 100,000 population.(1)Worldwide incidence of JIA ranges from 0.8-22.6/100,000 children per year and prevalence ranges from 7-401/100,000.(2) These wide ranging numbers are probably a reflection of population differences, environmental exposure and immunogenetic susceptibility, along with

variations in diagnostic criteria. Various studies showed that the rates of JIA were low in Asian population(3,4) and higher frequencies of JIA were noted in those of European descent(5,6). Different epidemiological studies have reported a wide variance in incidence and prevalence in different parts of the world. This might be attributed to various classification systems used in different studies, differences in data ascertainment or study design, difficulty in making accurate diagnosis and also because of paucity of trained pediatric rheumatologists worldwide.

Thierry et al conducted a systematic literature review to identify the incidence and prevalence of JIA in Europe. They concluded that incidence rates varied from 1.6 to 23 and prevalence from 3.8 to 400/100,000. (7)Oligoarticular JIA was found to be the most frequent form with pooled incidence rate 3.7 [3.5–3.9] and prevalence 16.8 [15.9–17.7]/100,000). In Europe in 2010, the estimated number of incident cases was 6896 [5481–8578] and 59,175 [44,256–76,983] prevalent cases.(7)

Berntson et al showed that that the incidence of JIA in Nordic(Denmark, Finland, Iceland ,Norway and Sweden) countries was 15 per 100,000 children per year.(8) A meta-analysis done on various published epidemiological studies in JIA found that the prevalence ranged from 12 per 100,000 children in clinic-based studies to 132 per 100,000 children in population-based studies.(9)

Accurate data on the incidence and prevalence of JIA in India is currently lacking.A study done in India by Vishwanathakumar et al to study the clinical spectrum of JIA in a tertiary referral hospital, showed that polyarticular JIA was the commonest subtype

followed by pauciarticular JIA. In this hospital based study, 112 children with JIA were enrolled and 8.9% had systemic onset JIA,55.3% had polyarticular JIA and 35.7% had pauci articular JIA.(10)

The incidence of JIA is more in girls than in boys, although the gender distribution varies with different subtypes(11). For example, the gender ratio is 2:1(females to males) in the Oligoarticular, polyarticular and psoriatic subtypes whereas the ratio is more equal in systemic JIA. In contrast more number of boys are affected in enthesitis related arthritis(12). Girls are found to be more affected in the subgroup associated with uveitis(13).

## CLASSIFICATION:

Various nomenclature and classification systems have been proposed for childhood arthritis in the last 40 years.(14,15) The latest and widely accepeted classification system currently is that put forward by the International League Of Associations For Rheumatology in 1997 and revised in 2001. ILAR criteria divides JIA into 7 subtypes namely: namely Oligoarticular arthritis, rheumatoid-factor [RF]- positive polyarthritis, RF-negative polyarthritis, systemic-onset JIA, psoriatic arthritis, enthesitis-related arthritis and undifferentiated arthritis.(14)

## CLASSIFICATION OF CHRONIC ARTHRITIS OF CHILDHOOD:

**Table 1:**

| Organisation | EULAR (European League Against Rheumatism) | ACR (American College of Rheumatology) | ILAR (International League of Association For Rheumatology) |
|---|---|---|---|
| Criteria Name | Juvenile Chronic Arthritis | Juvenile Rheumatoid Arthritis | Juvenile Idiopathic Arthritis |
| Year | 1977 | 1986 | 1997,revised 2001 |
| Age of Onset | < 16 years | < 16 years | < 16 years |
| Duration | ≥ 3 months | ≥ 6 weeks | ≥ 6 weeks |
| Subsets | 1.Pauciarticular <5 joints<br>2. Polyarticular: .4 joints, RF-<br>3. Systemic: arthritis with characteristic fever<br>4. Juvenile rheumatoid arthritis: .4 joints, RF+<br>5. Juvenile ankylosing spondylitis<br>6. Juvenile psoriatic arthritis | 1. Polyarthritis: ≥5 inflamed joints<br>2. Oligoarthritis (pauciarticular disease): <5 inflamed joints<br>3. Systemic onset: arthritis with characteristic fever | 1. Systemic<br>2. Oligoarthritis<br>a. Persistent<br>b. Extended<br>3. Polyarthritis: RF-<br>4. Polyarthritis: RF+<br>5. Psoriatic arthritis<br>6. Enthesitis-related arthritis<br>7. Undifferentiated<br>a. Fits no other category<br>b. Fits more than one category |

Abbreviation : RF: Rheumatoid Factor

**Table 2:**Criteria for the Classification of Juvenile Rheumatoid Arthritis

| |
|---|
| Age at onset: <16 yr<br>Arthritis (swelling or effusion, or the presence of 2 or more of the following signs: limitation of range of motion, tenderness or pain on motion, increased heat) in ≥1 joint<br>Duration of disease: ≥6 wk<br>Onset type defined by type of articular involvement in the 1st 6 mo after onset:<br>Polyarthritis: ≥5 inflamed joints<br>Oligoarthritis: ≤4 inflamed joints<br>Systemic-onset disease: arthritis with rash and a characteristic quotidian fever<br>Exclusion of other forms of juvenile arthritis |

*Modified from Cassidy JT, Levison JE, Bass JC, et al: A study of classification criteria for a diagnosis of juvenile rheumatoid arthritis, *Arthritis Rheum* 29:174–181, 1986.

## ETIOLOGY:

The etiology is unknown. It is postulated to be multifactorial and appears to differ from one subset to another. It is considered to be an auto inflammatory disease(13). In conditions such as Oligoarthritis (antinuclear antibodies) and rheumatoid factor positive polyarthritis (IgM rheumatoid factor) where autoantibodies are common, humoral immune system seems to be central in the pathogenesis of the disease. In contrast, Enthesitis related arthritis , rheumatoid factor-negative polyarthritis, and psoriatic arthritis have relatively less tendency to autoantibody formation but have strong association with polymorphisms at the histocompatibility locus(16). The fact that genetic factors form only a part of the etiology is evidenced by the fact that familial

arthritis is very rare, although not unknown. In Juvenile Idiopathic arthritis, the fundamental pathological process is chronic inflammation, in which both the innate and adaptive immune systems play critical roles. In all categories of JIA, products of activated T cells and macrophages are involved in pathogenesis of synovitis.

Various studies have also looked at environmental influences as a possible etiological factor. A Study done by Mason et al suggested that breastfeeding has a protective effect on the development of Juvenile Idiopathic Arthritis especially in Oligoarticular disease (17); however, a strong relationship was not confirmed in another study (18). A study done by Jaakkola et al suggested that maternal smoking during pregnancy is a risk factor for the development of arthritis in the first 7 years of life, especially in girls(19).In addition to diverse genetic predispositions, alterations in the immune responses, and different environmental triggers, the various theories of pathogenesis must also account various factors such as the heterogeneity of the disease; the increase in prevalence of oligoarthritis, polyarthritis, and psoriatic arthritis in girls, as against the higher incidence of enthestis related arthritis in boys; also of note is the narrow peak ages at onset for some types of arthrtis such as oligoarthritis, in contrast to the absence of a peak age at onset for systemic disease; and the association of extraarticular complications such as uveitis in certain disease subsets. Thus we can conclude that there may be multiple etiological events, or that the disorder may result from a single pathogenic vector which manifests itself in diverse clinical patterns based on its interactions with the host. It may be postulated that an environmental factor affecting a child with a specific genetic

predisposition, at a point of vulnerability which may be defined by age, intercurrent illness, hormonal abnormalities, psychological stress , immunological maturity, prior antigenic experience and trauma results in a clinical disorder. It is a complex interaction between all the above mentioned factors which leads to the causation of the disease. (13)

## PATHOGENESIS:

JIA is an autoimmune disorder and is associated with alterations in humoral and cell-mediated immune systems. T lymphocytes play a central role in the pathogenesis by releasing pro-inflammatory cytokines favoring a type 1 helper T-lymphocyte response. In JIA, there is also an increase in the recruitment of T lymphocytes specific for synovial non–self antigens.

B-cell activation, immune complex formation, and complement activation also promote inflammation. Systemic disease and more severe articular disease may occur as a result of upregulation of certain inflammatory networks due to the inheritance of specific cytokine alleles by the affected individual.(2)

Systemic Onset Juvenile Idiopathic arthritis is characterized by dysregulation of the innate immune system and a lack of autoreactive T cells and autoantibodies. It is thus classified as an auto inflammatory disorder.

The complex interaction between various immunologic abnormalities, genetic predisposition coupled with environmental triggers cause inflammatory synovitis which

is characterized pathologically by villous hypertrophy and hyperplasia with hyperemia and edema of the synovial tissue. There is also presence of vascular endothelial hyperplasia which is characterized by infiltration of mononuclear and plasma cells with a predominance of T lymphocytes. In advanced and uncontrolled disease, pannus formation and progressive erosion of articular cartilage and contiguous bone occurs (2).

## VARIOUS SUBTYPES OF JIA:

### OLIGOARTICULAR JIA:

Oligoarticular juvenile idiopathic arthritis (JIA) is defined as a chronic inflammatory arthritis of unknown origin that has onset before the age of 16 and persists for at least 6 weeks.It is further divided into persistent and extended Oligoarticular JIA.The term persistent is used if no more than four joints are affected during the disease course and the term extended is used if, after the initial 6-month period, the total number of affected joints exceeds four. The International League of Associations for Rheumatology (ILAR) classification also requires that patients who otherwise fulfill these criteria be excluded from the category if the patient has psoriasis, or if there is a history of psoriasis or a disease associated with the human leukocyte antigen (HLA) allele HLA-B27 in a first-degree relative; if the disease began in a male older than 6 years of age; or if two positive tests for rheumatoid factor (RF) were obtained at least 3 months apart. (20)

The peak incidence of oligoarthritis is between 1 and 3 years of age and Oligoarticular JIA is the most common cause of chronic oligoarthritis in girls younger than 6 years of age .The first 6 months of disease is characterized by inflammation in four or fewer joints. Children are not usually systemically ill, and, except for chronic uveitis, extraarticular manifestations are distinctly unusual. In oligoarthritis, the affected joint is swollen and often warm, but usually not very painful or tender, and almost never red. Varying degrees of effusion may be present. Oligoarticular JIA is predominantly a disease of the lower extremities.In at least half of the reported cases, only a single joint is affected (monoarticular onset), usually the knee. Uveitis may be present at onset of the disease; it eventually affects up to 20% of children and is usually asymptomatic.

Laboratory indicators of inflammation may be normal in children with oligoarthritis, although mild to moderate elevation of acute phase reactants (ESR and CRP) may occur. Blood counts are usually normal. Tests for rheumatoid factor is almost always negative, although occasionally children with a single affected joint (often the wrist) have rheumatoid factor. ANA is positive in low to moderate titer (1 : 160 to 1 : 640) in 62% to 65% of children with oligoarthritis, particularly in girls and in those with uveitis(21).
Antibodies to histones are present in 6% of children with Oligoarticular JIA and 12% in those with uveitis(22).Antibodies that react with citrullinated peptides have rarely been demonstrated in children with oligoarthritis

POLYARTICULAR JIA:

Chronic childhood arthritis affecting 5 joints or more in the first 6 months of disease is defined as polyarthritis.The International League of Associations for Rheumatology (ILAR) classification system for juvenile idiopathic arthritis (JIA)2 further categorizes polyarthritis as rheumatoid factor (RF) negative if tests for RF are negative, and RF positive if RF is detected on two occasions at least 3 months apart(20). Polyarthritis is present in 20% of JIA patients; of these, approximately 85% have negative tests for rheumatoid factor. RF-negative polyarticular JIA can begin at any age before 16 years, but onset age displays a biphasic trend with a peak at ages 1 to 3 years and another peak between childhood and adolescence.(23)Girls are four times more frequently affected than boys in RF-negative polyarthritis. The mean age at juvenile onset of RF-positive polyarthritis is 9 to 11years, and the range is 1.5 to 15 years (13) .

Joint disease predominates in children with RF-negative polyarticular JIA and extraarticular features are infrequent. Wrists and ankles are most commonly affected in these children and small joint involvement of the hands or feet usually occurs.The temporomandibular joint (TMJ) is commonly affected in children with a polyarticular disease course regardless of onset subtype. Systemic manifestations in seronegative polyarticular JIA are unusual and if present can include fatigue and growth failure. Fever seldom occurs and when present is low grade. ANA can be present in approximately half of the patients with RF negative polyarticular JIA.

SYSTEMIC ONSET JUVENILE IDIOPATHIC ARTHRITIS:

Systemic arthritis is definedby the ILAR classification of juvenile idiopathic arthritis (JIA)based on the following criteria: presence of arthritis and a documented quotidian fever of at least 2 weeksduration plus one of the following: typical rash, generalized lymphadenopathy, enlargement of liver or spleen, or serositis. It can occur at any time during childhood with a peak age of onset between 1 and 5 years. Boys and girls are equally affected(13).

In systemic onset JIA there is profound activation of the innate immune system which results in elevated levels of several inflammatory cytokines such as IL-1, IL-6, IL-8, IL-18 and tumor necrosis factor, all of which play a major role in the pathogenesis. Systemic JIA is characterized by arthritis, fever, rash, and presence of visceral involvement such as hepatosplenomegaly, lymphadenopathy, and serositis such as pericarditis. Fever in systemic JIA is usually high grade and usually≥39°C (102.2°F) and occurs on a daily or twice-daily basis for at least 2 week, with a rapid return to normal or subnormal temperatures. The fever is often present in the evening and is frequently accompanied by a characteristic faint, erythematous, macular rash. Rash most commonly occurs on the trunk and proximal extremities but may also develop on the face, palms, or soles. The rash tends to be migratory, is usually non pruritic and is strikingly evanescent in any one area and disappears within a few hours and leaves no residua. Rash also exhibits koebner phenomena which is a cutaneous hypersensitivity reaction in which classic lesions are brought on by superficial trauma.

The cluster of fever, rash, hepatosplenomegaly, and lymphadenopathy is present in more than70% of affected children. Some children present initially with only systemic features and no arthritis. However, arthritis evolves over time and is essential for a definitive diagnosis. Arthritis may affect any number of joints. The course is classically polyarticular, may be very destructive, and can include hip, cervical spine, and temporomandibular joint.

The most dreaded complication of systemic onset JIA is the development of macrophage activation syndrome (MAS) and can occur at any time during the disease course. MAS is also referred to as secondary hemophagocytic syndrome or hemophagocytic lymphohistiocytosis (HLH). The predisposition of individuals with systemic JIA to develop MAS may be associated with cytolytic pathway defects and is usually linked to heterozygous mutations in some of the genes associated with primary HLH such as *PRF1*, *MUNC13-14*, *LYST*, and *STXBP2*, as well as to high levels of proinflammatory cytokines such as IL-6. (13)

MAS is characterized clinically by the rapid development of high unremitting fever, hepatosplenomegaly, lymphadenopathy, hepatic dysfunction, encephalopathy and skin and mucosal bleeding manifestations. Patients can develop multiorgan involvement and may progress to respiratory distress, renal failure, disorientation, seizures, and reducedlevel of consciousness, hypotension, and shock. Macrophage activation syndrome is associated with high mortality and morbidity and requires prompt treatment(2).

Laboratory studies may show low hemoglobin levels with thrombocytopenia. Typically, liver enzymes, lactate dehydrogenase (LDH), triglycerides, and ferritin levels are elevated. Extreme hyperferritinemia with levels above 10,000 μg/L is characteristic, and serum albumin is low. D-dimers levels are elevated and prothrombin time (PT) and the partial thromboplastin time (PTT) may be prolonged. The erythrocyte sedimentation rate (ESR) may drop sharply in association with hypofibrinogenemia, but C-reactive protein (CRP) is typically elevated. Demonstration of hemophagocytosis in the bone marrow or other tissues is diagnostic, but MAS may occur in the absence of demonstrated tissue hemophagocytosis in up to 40% of patients. The features that were decided by an international consensus panel as the most important indicators of MAS include a falling platelet count, extreme hyperferritinemia, evidence of macrophage hemophagocytosis in the bone marrow, increased liver enzymes, falling leukocyte count, persistent, continuous fever ≥38°C (100.4°F), falling ESR, hypofibrinogenemia, and hypertriglyceridemia.

ENTHESITIS RELATED ARTHRITIS:

It is predominantly a disease affecting joints and entheses of the lower extremities and can eventually affect the spine or sacroiliac (SI) joints. It is characterized by the absence of rheumatoid factor (RF) and by a strong association with the human leukocyte antigen–B27 (HLA-B27). ERA affects 11–16% of children with JIA. In the past this type of JIA was called juvenile ankylosing spondylitis, seronegative enthesopathy and arthropathy (SEA) syndrome, or undifferentiated juvenile spondylarthropathyThe mean age at

diagnosis is around 10 to 13 years (range 2.8 to 17.6 years) and boys are more affected than girls.

Enthesitis is defined as localized inflammation at the point of insertion of tendons, ligaments, joint capsules, or fascia to bone (11). The typical locations for enthesitis are in the lower limbs and areas commonly involved are the iliac crest, posterior and anterior superior iliac spine, femoral greater trochanter, ischial tuberosity, patella, tibial tuberosity, Achilles, and plantar fascia insertions. ERA should be considered in patients presenting with significant heel or foot pain. Another unique feature of this subtype of JIA is the involvement of the axial skeleton, especially in the sacroiliac joints, with some children developing ankylosing spondylitis within 10 to 15 years of disease onset.

## CLINICAL MANIFESTATIONS:

Constitutional Signs and symptoms:

Anorexia, weight loss, and growth failure occurs in many children with Juvenile Idiopathic arthritis. Children with polyarticular or systemic disease have significant fatigue, especially at disease onset and during periods of poor disease control. Fatigue may be manifested as an increased sleep requirement, lack of energy, or increased irritability. Night pain may be present although it is more characteristic of malignancy. However, night pain can interrupt sleep and contribute to fatigue.

Anorexia may occur as a result of gastric irritation secondary to nonsteroidal antiinflammatory drug use, or nausea secondary to methotrexate use.

Growth retardation may occur as a consequence of active JIA and can also be attributed to prolonged corticosteroid use. Puberty and secondary sexual characteristics are often delayed in children with active inflammation.

Pain and stiffness:

A child with chronic arthritis may not complain of pain at rest. However, if the joint is inflamed, active or passive motion of a joint elicits pain especially at the extremes of range of motion. Pain can be mild, moderate or rarely severe in severity and is usually described as an aching or stretching type of pain. Pain elicited by pressure (tenderness) is maximal at the joint line.Bone pain or tenderness is not a characteristic feature of arthritis, and if it is present, the possibility of an infection or malignancy involving the bone should be considered.

Joint stiffness is a characteristic symptom exhibited by most children with Juvenile Idiopathic arthritis. Joint stiffness is more profound in the morning particularly on arising from bed or after prolonged inactivity. Parents usually describe stiffness as slowness or awkwardness of the gait which is most marked in the morning or after a nap or prolonged sitting and which improves with activity or heat application to the affected area. Stiffness which lasts for more than 15 minutes signifies a considerable level of joint inflammation.

Features of Inflamed Joint:

The cardinal signs of inflammation that actively inflamed joint exhibits are: Swelling, pain, heat, loss of function, and erythema. Swelling of a joint usually occurs as a result of edema of the periarticular soft tissue or because of intraarticular effusion, or from hypertrophy of the synovial membrane. Large synovial cysts are unusual and may occur in the antecubital area or anterior to the shoulder. When they occur in the popliteal space, it is called a baker cyst.

Inflamed joints in children with juvenile idiopathic arthritis areoften warm but almost never erythematous. In contrast, joints in septic arthritis or acute rheumatic fever will be erythematous.There is limitation of range of movements, particularly in extension,because this is usually a position of relative comfort, accommodating shortened soft tissue structures. Hyperextension is characteristicallythe first range of movement to be lost in the cervical spine. Similarly, extension or hyperextension is first lost in elbows, wrists, metacarpophalangeal and interphalangeal joints, but flexion range can also be diminished.

The hallmark symptom of intraarticular hip joint disease is loss of internal rotation and flexion. Loss of hyperextension or extension, and sometimes flexion occurs if the knees are affected. Loss of ankle dorsiflexion, subtalar eversion, and midfoot supination are characteristic of an inflamed joint.

Ocular Involvement:

Ocular inflammation may occur at any time in the course of Juvenile Idiopathic Arthritis. Uveitis is characteristically asymptomatic in most cases, except in enthesitis related arhritis, where it is characterized by a painful pink eye. Keratoconjunctivitis sicca occasionally occurs, particularly in patients with rheumatoid factor-positive polyarthritis

Distribution Of affected Joints:

Any joint can be affected in Juvenile Idiopathic arthritis. Most frequently involved joints are the large joints such as knees, ankles, wrists, elbows and hips. Small joints of the hands and feet can also be affected, especially in cases of polyarticular-subtype of disease. Disease in the apophyseal joints of the cervical spine are rare and occurs at onset in only 2% of children. These children may present with torticollis.The acromioclavicular, sternoclavicular, and sternomanubrial joints are
infrequently affected.

Various subtypes of Juvenile Idiopathic Arthritis have different characteristic patterns of joint involvement. In, polyarticular disease, symmetrical involvement of large and small joints is typical. Patients with Enthesitis related Arthritis (ERA) have predominantly involvement of the joints of the lower extremity. The presence of hip joint disease is not uncommon in ERA, but rarely occurs in Oligoarticular JIA. Patients with Psoriatic arthritis have a somewhat asymmetrical involvement and involves both large and small joints, sometimes including the distal interphalangeal joints.

## LABORATORY FEATURES:

Laboratory parameters are used toprovide support for a diagnosis of chronic arthritis. No laboratory test or combination of studies can confirm the diagnosis of Juvenile Idiopathic arthritis. Laboratory parameters can be used to provide evidence of inflammation, to monitor toxicity of therapy, and as a research tool to understand more completely the pathogenesis of the disease

Hematological Indices:

Hematologic abnormalities often reflect the degree of systemic or articular inflammation. Children with involvement of only a limited number of joints usually do not exhibit any hematological aberrations beyond that of mild anemia.Normocytic hypochromic anemia is usually found in children with moderately extensive arthritis. In children with severe, uncontrolled disease, the anemia may be severe with hemoglobin in the range of 7 to 10 g/dL. The cause for anemia in these children is due to chronic disease; however, iron deficiency may also play a role as plasma iron transport and iron available for erythropoiesis are decreased in systemic disease.

Leukocytosis is usually found in children with active juvenile idiopathic arthritis in all disease subtypes. Values are strikingly high in children with systemic- onset disease and are usually in the range of 30,000 to 50,000 to cells/mm3 (30 to 50 × 109/L). Thrombocytosis is commonly found and the platelet count may rise dramatically in

children who have long standing disease or those with severe disease. Thrombocytosis can also occur during disease exacerbation. Thrombocytopenia is rare and may signal an evolution of the disease into systemic lupus erythematosis, drug toxicity, or the development of macrophage activation syndrome.

In the setting of Macrophage activation syndrome, all cell lines such as hemoglobin, white blood cells and platelets have the propensity to decline precipitously owing to the consumptive process. A low or normal white blood cell count along with a dropping platelet trend in a child with active systemic onset juvenile idiopathic arthritis should raise the concern of macrophage activation syndrome.

ACUTE PHASE REACTANTS:

Acute phase reactants such as ESR and CRP are elevated in children with juvenile idiopathic arthritis. Erythrocyte sedimentation rate (ESR) reflects the plasma fibrinogen level and is a useful measure of disease activity at onset of disease and during follow-up of a child with arthritis. Similarly, C-reactive protein (CRP) level is also a reliable marker of inflammatory response.

The third component of complement (C3) isan acute phase protein and is often elevated in the sera of children with active disease. The activated form of the molecule (C3d) may be increased as well and is an indicator that complement-mediated tissue damage plays a role in the pathogenesis of arthritis in children.

Ferritin is an acute phase reactant which can be grossly elevated in children with arthritis. Its values are strikingly high and is usually more than 10,000 ng/ml in those with macrophage activation syndrome.

Serum immunoglobulin levels are also increased in children with arthritis and its levels correlate with disease activity. Extreme hypergammaglobulinemia can bepresent in very sick children and returns toward normal with clinical improvement.

Synovial fluid from patients with juvenile idiopathic arthritis is usually inflammatory. The main components of the synovial fluid in inflamed joints are polymorphonuclear neutrophilsand mononuclear cells, including lymphoid dendritic cells.As in adult rheumatoid arthritis,synovial fluid levels of glucose may be low. Likewsie, complement levels are not as uniformly depressed as inadult disease. The concentration of glycosaminoglycans such as hyaluronic acid and chondroitin sulfates in synovial
fluid is decreased compared with normal controls, accounting for the low viscosity of inflamed synovial fluid.

Radiologic Features:

Early radiographic features of arthritis include soft tissue swelling, periarticular osteopenia, and periosteal new-bone apposition around joints that are affected. Continuation of active disease may lead to subchondral erosions, loss of cartilage,

varying degrees of bony destruction, and fusion. Characteristic radiographic changes in cervical spine aremost frequently located in the neural arch joints at C2-C3 and may progress to atlantoaxial subluxation. MRI is the imaging of choice to detect early changes.

## TREATMENT:

PHARMACOLOGIC THERAPY:

Juvenile Idiopathic arthritis is a disorder that cannot yet be cured. However, control of the disease is achievable with medications. The aim of treatment is to induce disease remission, and in the process to control pain and preserve range of motion, strength of the muscle and its function. Therapy is also used to manage systemic complications; and to facilitate normal nutrition, growth, and physical and psychological development. Most children with JIA often are in need of a therapy which is a combination of pharmacological, physical, and psychosocial approaches. Patients with JIA need individualized treatment plans, and therapy is modified based on disease subtype and severity, presence of poor prognostic indicators, and response to medications. Monitoring for potential drug toxicities is also an intrinsic part of disease management.

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been the traditional initial approach in the management of children with Juvenile Idiopathic arthritis. Those children with oligoarthritis subtype often show only partial response to nonsteroidal

antiinflammatory drugs (NSAIDs), with improvement in inflammation and pain .Thosewho have functional limitations, such as joint contracture or leg-length discrepancy and those who have no or partial response after 4-6 wk of treatment with NSAIDs often benefit from injection of intraarticular corticosteroids. Triamcinolone hexacetonide is a long-lasting medication that is often used and provides response for a prolonged period of time. There will be a minority of patients with Oligoarticular subtype of JIA who would not respond to NSAID's as well as intraarticular injections. This minority of patients require treatment with disease-modifying antirheumatic drugs (DMARDs), methotrexate,

and, if no response, then TNF inhibitors.

Children with polyarthritis and systemic onset JIA are usually more symptomatic and NSAIDs alone rarely induce remission in them. DMARD which is the oldest and least toxic which is available for adjunctive therapy is Methotrexate. It usually takes 6 to 12 weeks to exhibit its effects. If monotherapy with Methotrexate fails, then, it warrants theaddition of a biologic DMARD. Biological medications are drugs which inhibit proinflammatorycytokines, such as TNF-α, IL-1, and IL-6 and have excellent disease control. TNF-α antagonists such as Etanercept and Adalimumabare used to treat children with an inadequate response to methotrexate and thosewith poor prognostic factors, or severe disease onset. Commencement of early aggressive therapy with a combination of methotrexate and aTNF-α antagonist may result in the achievement of disease remission earlier than usual.

Systemic symptoms predominate in children with systemic onset JIA and TNF inhibition is not as effective for the control of the same. Systemic steroids are started when systemic symptoms predominate followed by the initiation of IL-1 or IL-6 antagonist therapy, which often induces a dramatic and rapid response. That subset of patients with severe disease activity may directly have to be started on treatment with Anakinra. Two FDA-approved drugs which can be used for treatment of children with systemic onset JIA older than 2 years of age are Canakinumab which is an IL-1β inhibitor, and tocilizumab which isan IL-6 receptor antagonist.

The use of systemic corticosteroids can often be avoided or minimized with the advent of newer DMARDs. Systemic steroids should be used only for the management of severe systemic illness and as a bridge therapy while awaiting therapeutic response to a DMARD, and for control of uveitis. Use of high dose steroids is associated with severe side effects such as Cushing syndrome, growth retardation, and osteopenia, and they cannot prevent joint destruction.

Another class of drugs known as Oral Janus kinase (JAK) inhibitors such as tofacitinib, and ruxolitinib inhibit JAK signaling pathways involved in immune activation and inflammation. Tofacitinib is an FDA approved medication for adults with rheumatoid arthritis.

Asymptomatic uveitis is often found in children with JIA periodic slit-lamp ophthalmologic should form a part of the management process to monitor for the same. Optimal management of uveitis involves a collaborative effort between the

ophthalmologist and rheumatologist. Initial treatment of uveitis is with mydriatics and corticosteroids which can be used topically, systemically, or through periocular injection. Methotrexate and antibodies to TNF-α (adalimumab and infliximab) are effective in treating severe uveitis.

NUTRITION AND OCCUPATIONAL THERAPY:

Various studies have documented different degrees of undernutrition or obesity in children and adolescents with JIA.Assessment of nutritional status should be an integral component of every patient's evaluation.Retardation of growth and impairment of bone mineralization invariably occur during periods of active disease and are exacerbated by glucocorticoid administration, anorexia, or inanition. Supplementation of calcium, vitamin D, and folic acid is oftenindicated in children undergoing treatment for JIA.

Physical therapy and occupational therapy are invaluable adjuncts to any treatment program and its objectives are to minimize pain, maintain and restore function, and prevent deformity and disability. Treatment team should also ideally include a social worker and nurse clinician who act asimportant resources for families, to recognize stresses imposed by a chronic illness and to identify appropriate community resources, and also aid compliance with the treatment protocol.

## BIOMARKERS IN JUVENILE IDIOPATHIC ARTHRITIS:

A biomarker is a molecule which can be easily measured in accessible patient material such as blood, urine or saliva, and is ideally obtained using a relatively non-invasive approach. The components used as a biomarker should ideally be stable over time within the patient sample and should be able to be measured by an accurate, easy to use and reproducible assay and at an affordable cost to the patient as well as the health care providers.(24) .An ideal biomarker for pediatric use would be one whose normative values are not affected by age related development in children, thus avoiding the need for age specific normal-range data sets. Biomarkers are being increasingly used in various areas of clinical practice for assessment of disease activity and to help aid in the choice of treatment and for prognostication. However, most of these studies are done in adults and the data from these studies are extrapolated to children without considering differences in disease pathogenesis, age dependent changes in reference values owing to growth and development in children over time, effect of ontogeny on disease evolution and response to treatment, and changes in phenotypic gene expression.(25, 26)

Use of biomarkers in JIA is a rapidly expanding field as pediatric rheumatologic diseases present inherent challenges in initial diagnosis and continued monitoring of disease activity. At disease onset, characteristic symptoms may take time to accumulate, and diagnoses are frequently of exclusion. Patients then may go on to have subclinical inflammation or relapsing disease. The scope of pediatric biomarkers in JIA is huge but

despite this, there are no validated pediatric biomarkers available to aid in setting up a cornerstone on which disease activity can be predicted or drug choice can be based.

JIA is a heterogeneous group of disorders which uses both clinical findings and some biomarkers to divide cases into its various subtypes. Polyarticular form of JIA which involves five or more joints in the first six months of disease is divided into its 2 subtypes (Rheumatoid factor positive and rheumatoid factor negative) based on the presence or absence of a serum autoantibody called rheumatoid factor (RF). These 2 clinical subtypes are in turn different from each other with respect to their genetics, age of onset and prognosis.(27,28). Similarly, the presence of positive serum Anti-Nuclear Antibody (ANA) has been associated with an increased risk of chronic anterior uveitis(29), which, if not screened properly and treated, can result in permanent visual loss.(30, 31) Thus, both these antibody biomarkers (RF and ANA) can be easily measured in serum and currently are part of routine clinical care in management of children with JIA.

A study done by Gilliam et al in children with JIA measured the levels of a panel of biomarkers which included cartilage oligomeric matrix protein (COMP), anti-cyclic citrullinated peptide (anti-CCP) antibodies, ), IgM rheumatoid factor (RF), IgG RF, and IgA RF, C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR). They found significantly elevated levels of IgA RF, IgM RF, and anti-CCP antibodies in patients with joint erosions and joint space narrowing which are markers of aggressive disease. COMP

levels were elevated in early course of disease whereas ESR, CRP, and IgA RF were significantly elevated in patients with active synovitis. (32)

Ferritin is a biomarker that is elevated in conditions associated with acute inflammation such as rheumatoid arthritis and systemic lupus erythematosis. In these conditions, it usually rises in parallel with other acute phase reactants. However, in children with systemic onset Juvenile idiopathic arthritis (SOJIA), ferritin levels are noted to be extremely high and exceed 1000 ng/ml whereas the normal range of ferritin is between 7-140 ng/ml (33,34) and thus may be helpful in establishing a diagnosis of systemic onset Juvenile idiopathic arthritis (SOJIA). Also to be noted is the fact that whenever SOJIA is associated with macrophage activation syndrome, ferritin levels are strikingly high and usually exceeds 10,000 ng/ml, thus acting as a good biomarker for diagnosis of SOJIA and macrophage activation syndrome.

Interleukin 18 (IL 18) is a unique cytokine which belongs to Interleukin 1 family. It is present in epithelial cells, blood monocytes and keratinocytes. Interleukin 18 induces production of Interferon gamma by Natural killer cells and T cells and also induces production of Tumor necrosis alpha and chemokine secretion by macrophages. Normal levels of circulating IL 18 in the serum of healthy individuals are 100 pg/ml. Its levels are elevated in conditions associated with systemic inflammation such as SOJIA (35). In systemic onset juvenile idiopathic arthritis, the levels of interleukin 18 are elevated out of proportion as compared to other cytokines. In conditions where SOJIA is complicated by Macrophage activation syndrome, levels of circulating IL18 are elevated more than

tenfold. Studied have also proven that levels of circulating IL 18 correlates strongly with disease activity and was also found to be useful in predicting an upcoming flare of the disease(36).

## CALPROTECTIN:

Calprotectinis a calcium- and zinc-binding protein of the S100/calgranulin family (37).S100 proteins constitute a family of acidic calcium-binding proteins thatare important in intracellular calcium metabolism. (38). In 1983, Dale et al originally discovered Calprotectinas an antimicrobial protein that was present in the cytoplasm of neutrophil granulocytes(39) . Later on, Sander et al and Roth et al described it as a promising marker of inflammation(40). Srikrishna et al, in their study, proved that the molecule is involved in the recruitment of inflammatory cells by interactions with endothelial cells(41).

Calprotectin is also known in literature by other synonyms such as S100A8/A9, MRP8/14 (myeloid-related protein), calgranulin A/B, 27E10 antigen, cystic fibrosis antigen,and L1 protein and myeloid-histiocyte antigen. Calprotectin is mainly found in the cytoplasm of neutrophils (42). Its concentration in neutrophils is so abundant that it constitutes about half of total cytosolic protein(43). It can also be expressed on activated monocytes and macrophages and can be produced by bonemarrow cells, squamous epithelium (keratinizing and non-keratinizing), some mucosal epithelial cells, micro

vascular endothelial cells and fibroblasts.The normal level is in the range 1–6 mg/l, and it increases in response to various tissue injuries and inflammation(44).

The term Calprotectin is used to describe a complex of two calciumbinding proteins of the S100 family, S100A8 and S100A9. The human S100A8 protein (also known as myeloid-related protein 8, calgranulin A, or L1 light chain) is made up of 93 amino acids and has a molecular weight of 10.8 kd. The human S100A9 protein (MRP-14, calgranulin B, and L1 heavychain) consists of 114 amino acids with a molecular weight of 13.2 kd. Hence, Calprotectin is a 24kDa heterodimer composed of one light (MRP8) and two heavy (MRP14).Calprotectin also contains zinc-binding domains, with a zinc-binding capacity higher than other S100 proteins, and are not affected by the binding of calcium.Calprotectin also has antibacterial activity due to the presence of these histidine based zinc-binding sequences (His-X-X-X-His motif) (42). It can be identified as a monomer, with separate chaining, or as a hetero- or homodimeric, trimeric or tetrameric complex (45). The genes for Calprotectin are located on the human chromosome 1q21 (37).

## FECAL CALPROTECTIN:

Calprotectin is a small calcium-binding protein belonging to the S100 family of proteins and contributes approximately 60% of the protein content of the cytosol in neutrophils. Polymorphonuclear neutrophils migrate to the intestinal mucosa from the circulation if

there is active intestinal inflammation. Any disturbance to the mucosal architecture due to the inflammatory process, results in leakage of neutrophils along with Calprotectin into the lumen leading to its subsequent excretion in feces. Calprotectin appears to be distributed homogeneously in feces and is stable for up to 7 days at room temperature. Multiple studies have hence looked at the usefulness of Calprotectin as a marker of intestinal inflammation.

Inflammatory bowel disease (IBD) involves chronic inflammation of the digestive tract and consists of 2 distinct clinical entities called ulcerative colitis and crohns disease. FecalCalprotectin has been proposed as a useful biomarker for the differential diagnosis between IBD patients and healthy controls. A study done by Moein et al showed that fecalCalprotectin levels were increased in patients with inflammatory bowel disease compared to healthy controls ($p<0.05$). Fecal Calprotectin had a stronger correlation with disease endoscopic activity than conventional inflammatory markers ($r=0.847$ versus $r= -0.44$ for CRP and $r=0.054$ for ESR in Crohn's disease and $r=0.798$ versus $r=0.463$ for CRP and $r=0.467$ for ESR in ulcerative colitis). Receiver operating characteristic (ROC) curve analysis showed fecal Calprotectin has larger area under the curve than conventional inflammatory markers (1 versus 0.849 for CRP and 0.846 for ESR)(46).

## CALPROTECTIN IN RHEUMATOLOGICAL DISORDERS:

The usefulness of Calprotectin as a biomarker in rheumatological disorders in an area of active research. Currently, many studies have suggested that Calprotectin is a potentially more sensitive marker of disease activity in rheumatoid disorders than conventional inflammatory markers such as the erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), because it directly reflects inflammation in the synovium (47). Multiple studies have shown that alterations of Calprotectin level are associated with disease activity in patients with Still's disease (48), rheumatoid arthritis (RA)(47), primary Sjögren's syndrome (pSS) (49), ankylosing spondylitis (AS) (50), psoriatic arthritis (PsA) (51), juvenile idiopathic arthritis (JIA) (52) and systemic lupus erythematosus (SLE) (53).

Garcia et al conducted a study on 60 patients with rheumatoid arthritis study with the objective to evaluate relationships between serum Calprotectin levels, disease activity, and response to treatment. Calprotectin was also investigated as a predictive marker of clinical response. They found that Calprotectin levels correlated with rheumatoid factor levels ($r = 0.25$; $p < 0.05$). In their studyCalprotectin levels at baseline were not predictive of response to treatment but significantly decreased during treatment in responders ($p < 0.0001$). They concluded that Calprotectin levels correlated with clinical and laboratory assessments of joint inflammation and also decreased in response to treatment, indicating

that Calprotectin is a marker for assessment and monitoring of disease activity in patients with RA(47).

Similar studies to find the relationship between Calprotectin levels and rheumatoid arthritis were also carried out by Choi et al and Inciarte Mundo et al. Their studies focused on using Calprotectin as a marker of response to treatment with biological agents in patients with Rheumatoid arthritis. In their study, Choi et al recruited 170 patients with rheumatoid arthritis who were on treatment with adalimumab, Rituximab or infliximab and measures Calprotectin levels at baseline and also at 4 and 16 weeks after initiation of treatment. Patients were divided into clinical responders and non-responders. In their study they found that treatment with adalimumab, infliximab or rituximab lowered the level of Calprotectin significantly in the combined group of good and moderate responders, but not in non-responders. In those patients receiving adalimumab or infliximab, this change already reached statistical significance after 4 weeks of treatment, suggesting that Calprotectin could be used to monitor the early response to tumor necrosis factor α inhibitors (anti-TNF-α) treatment and to predict subsequent clinical response. In those receiving rituximab, a statistically significant decrease in Calprotectin level was found after 16 weeks of initiation of treatment in responders, but not in non-responders(54).

Inciarte-Mundo et al conducteda cross-sectional study on 87 patients with rheumatoid arthritis, receiving adalimumab, etanercept or infliximab for atleast 3 months and compared the accuracy of serum Calprotectin and acute-phase reactants such as C-

reactive protein (CRP) and erythrocyte sedimentation rate (ESR) in stratifying disease activity and to correlate Calprotectin levels with tumor necrosis factor inhibitor trough serum levels. They found that Calprotectin stratified disease activity in rheumatoid arthritis patients receiving TNF-α inhibitors more accurately than the acute-phase reactants such as CRP and erythrocyte sedimentation rate (ESR) and alsoCalprotectin level correlated inversely with TNF-α inhibitors trough serum levels (55). Inciarte-Mundo et al did another study to determine the association between Calprotectin, ESR, CRP and disease activity indices in 33 patients with rheumatoid arthritis receiving tocilizumab and found that serum Calprotectin level was higher in patients than in healthy controls. In their study, Calprotectin levels did not differ according to age or sex andCalprotectin level strongly correlated with all composite disease activity indices compared to ESR and CRP (56).

Guo et al studied the usefulness of Calprotectin in patients with adult onset still's disease (AOSD) and concluded that Calprotectin has a sensitivity of 63% and specificity of 80.1% for the diagnosis of adult onset still's disease. The positive rate of Calprotectin was significantly higher in AOSD cases compared to patients with other diseases (systemic lupus erythematosis, primary Sjogrens syndrome, rheumatoid arthritis etc.) and healthy controls ($p < 0.001$)(48).

Haga et al conducted a cross-sectional study of 100 patients with systemic lupus erythematosus (SLE) and found that Calprotectin levels were higher in patients than in matched controls (3661 micrograms/l versus 1051 micrograms/l; $P < 0.001$). They also

found that Calprotectin was the only laboratory parameter with significant association to the disease activity index SLEDAI ($r = 0.28$; $P < 0.01$). In their study, Calprotectin levels were higher in SLE patients with anti-DNA antibodies compared to patients without anti-DNA antibodies (4501 micrograms/l versus 3279 micrograms/l; $P = 0.01$) and those patients with arthritis were noted to have higher serum levels of Calprotectin than those without arthritis (7652 micrograms/l versus 2811 micrograms/l; $P < 0.01$) and hence indicating that the serum Calprotectin level is also indicative of arthritis activity in patients with SLE.(53). In 2016, Tantivitayakul et al studied the usefulness of Calprotectin (also known as MRP 8 or 14) and reported that myeloid-related proteins MRP8 and MRP14 significantly correlated with the early loss of the kidney function in SLE patients and with their therapeutic response. They concluded that MRP-8 and -14 can be used as non-invasive biomarkers to assess prognosis in patients with lupus nephritis(57). Tydén et al measured serum Calprotectin levels in 237 systemic lupus erythematosus patients with clinically inactive disease and found that elevated serum levels of Calprotectin in systemic lupus erythematosus (SLE) patients may be used as an indicator of severe cardiovascular disease. They also suggested that those SLE patients with elevated Calprotectin levels may benefit from more intense cardiovascular primary preventive strategies and introduction of early immunosuppressive treatment(58).

Serum Calprotectin levels were also studied in other rheumatologic conditions such as Behcets disease which is a form of systemic Vasculitis, Kawasaki disease and in polymyalgia rheumatica or temporal arteritis.Oktayoglu et al conducted a study on

patients with Behcets disease and concluded that even though serum Calprotectin levels were higher in patients with Behcets disease compared to healthy controls, it did not correlate well with other inflammatory markers such as ESR and CRP and also did not serve as a good marker for assessment of disease activity or quality of life in patients with Behcets disease(59).

Hirono et al studied the usefulness of Calprotectin as marker of disease activity and severity of coronary artery lesion development in patients with acute Kawasaki disease. They studied 61 patients with acute Kawasaki disease and concluded that serum Calprotectin levels as well as mRNA expressions of Calprotectin in granulocytes were strongly up regulated during the early stage of acute Kawasaki disease, and their levels decreased dramatically within 24 hours of intravenous immune globulin therapy ($p < 0.05$) in 45 responders. Furthermore, in 16 non-responders both of these increased after the initial treatment. They also found that the number of Calprotectin positive circulating endothelial cells was higher in patients with acute Kawasaki disease than in control patients, especially in those patients in whom coronary artery lesions developed(60).

Brun et al analyzed Calprotectin levels in patients with polymyalgia rheumatica and temporal arteritis and found that Calprotectin levels correlated well with other acute phase reactants and ESR and hence could be used as a marker of disease activity. They also found that Calprotectin levels decreased after starting oral steroids in these patients and its levels correlated with daily usage of prednisolone(61).

## CALPROTECTIN IN JUVENILE IDIOPATHIC ARTHRITIS:

Calprotectin is a good marker of inflammation and assessment of its usefulness as a marker of disease activity is an area of active research.

Bojko in his study compared bloodCalprotectinlevels in patients with systemic-onset, polyarticular, rheumatoid factor-negative and Oligoarticular subtypes of juvenileidiopathicarthritis (JIA). He also tried to find if any link existed between bloodCalprotectinlevels and clinical and laboratory markers of disease activity. Bojko found higher Calprotectin levels in patients with systemic-onset subtype of the disease (median 13,800 ng/ml), which differed significantly from levels in healthy children (median 1,800 ng/ml, $p$ = 0.00002), levels in patients with articular subtypes of JIA (median 2,700 ng/ml, $p$ = 0.000008), and patients with RF-negative polyarthritis (median 3,800 ng/ml, $p$ = 0.003226) and oligoarthritis (median 2,500 ng/ml, $p$ = 0.000009). Newly diagnosed patients with systemic onset juvenile idiopathic arthritis had the highest Calprotectinlevels, the median being 32,500 ng/ml (range: 13,800-177,000 ng/ml). Direct correlations were found between bloodCalprotectin and activity score ($p$ = 0.000009), ESR ($p$ = 0.000079) and CRP ($p$ = 0.000058)(62).

Systemic onset Juvenile Idiopathic arthritis is a disease that can mimic other conditions such as malignancies and infectious diseases and can pose a diagnostic challenge in the evaluation of pyrexia of unknown origin. Hence, Frosch at al studied the differences in Calprotectin levels in patients with systemic onset juvenile idiopathic arthritis,

malignancies and infectious diseases. They found that serum Calprotectin concentrations were significantly ($P < 0.001$) elevated in patients with active systemic-onset JIA (mean +/- 95% confidence interval 14,920 +/- 4,030 ng/ml) compared with those in healthy controls (340 +/- 70 ng/ml), patients with acute myeloblastic leukemia (840 +/- 940 ng/ml), patients with systemic infections (2,640 +/- 720 ng/ml), patients with acute lymphoblastic leukemia (650 +/- 280 ng/ml). Serum Calprotectin levels distinguished systemic-onset JIA from infections with a specificity of 95% and was found to be a better marker than C reactive protein and hence could be used as an excellent tool for the diagnosis of systemic-onset JIA, allowing early differentiation between patients with systemic-onset JIA and those with other inflammatory diseases(63).

Wittkowski et al(64) studied Calprotectin levels in children with juvenile idiopathic arthritis, systemic infections, malignancies (acute myeloblastic leukemia and acute lymphoblastic leukemia) andneonatal-onset multisystem inflammatory disease (NOMID). They found that mean levels of Calprotectin was much higher in children with juvenile idiopathic arthritis as compared to other above mentioned conditions and the sensitivity and specificity of Calprotectinto distinguish between systemic-onset JIA and infections were 66% and 94%, respectively(64).

Shenoi et al(65), in their study found that the median level of Calprotectin in patients with systemic-onset JIA was 38,600 ng/ml whereas it was 4,700 ng/mlin patients with a non-systemic disease course.Calprotectin levels were significantly elevated in systemic onset juvenile idiopathic arthritis with >80% sensitivity and >90% specificity(65).

Holzinger et al (66)not only looked at the usefulness of Calprotectin as a marker of disease activity in children with juvenile idiopathic arthritis, they also analyzed if it could be used to predict disease relapse. They found that serum Calprotectin levels increased significantly ($p<0.001$) (mean±95% CI 12.030±3.090 ng/ml) during disease flares compared with patients with inactive disease (864±86 ng/ml). During periods ofclinical remission, Calprotectinlevels of >740 ng/ml was able to predict disease flares accurately with a sensitivity of 92% and specificity of 88%. Calprotectin levels also correlated well with clinical disease activity, as assessed by physician's global assessment of disease activity ($r=0.62$), Childhood Health Assessment Questionnaire ($r=0.56$), active joint count ($r=0.46$) and with C-reactive protein ($r=0.71$) and erythrocyte sedimentation rate ($r=0.72$) (for all $p<0.001$)(66). Schulze et al conducted a similar study to assess the usefulness of Calprotectin to predict disease flare in children with Juvenile Idiopathic arthritis. Calprotectin levels in patients before relapses were significantly higher than the levels in patients in stable remission for one year (662 ng/ml versus 395 ng/ml; $p < 0.05$). Using a cut-off for Calprotectinof 450 ng/ml the likelihood ratio for relapse was 3.7 with a positive predictive value of 80% (67) .

Frosch et al(68) conducted a study to assess the usefulness of Calprotectin as a marker of inflammatory activity in children with pauci-articular juvenile idiopathic arthritis. . In their study, they found significantly higher concentration of Calprotectin in synovial fluid (mean 42,800 ng/ml) compared with serum (2,060 ng/ml). Calprotectin levels in serum correlated well with those in synovial fluid ($r = 0.78$) and showed a strong correlation

with disease activity (r = 0.62). In those patients who responded to therapy (triamcinolone injection), the serum concentrations of MRP8/MRP14 decreased significantly, whereas no differences were found in patients who showed no clinical benefit. The postulated reason for the above findings were that infiltrating neutrophils and monocytes within the inflamed joints strongly expressed Calprotectin which were specifically released during interaction of activated monocytes with tumor necrosis factor-stimulated endothelial cells and their Secretion was mediated via an increase in intracellular calcium levels in monocytes(68).

Only few Indian studies have looked at the role of Calprotectin in Juvenile Idiopathic arthritis. Rahman et al from Sanjay Gandhi post graduate Institute of medical sciences analyzed Calprotectin levels in patients with enthesitis related arthritis. In their study Median plasma levels of Calprotectin was 10 862.3 ng/ml which was much higher than controls (4426.1 ng/ml, $P < 0.0001$). Patients with active disease (11 669.5 ng/ml) had higher levels as compared with inactive disease (4421.8 ng/ml, $P < 0.0001$). They also noticed that plasma Calprotectin levels decreased on follow-up after 3 months only in patients who responded to treatment ($P = 0.012$) and Calprotectin levels were higher in synovial fluid as compared to plasma(69)

A study done by Walscheid at al analyzed if Calprotectin could be used as a marker to predict intraocular inflammation in children with juvenile idiopathic arthritis associated uveitis. SerumCalprotectinlevels were elevated in patients with juvenile idiopathic arthritis associated uveitis as compared to nonuveitic controls ($P< 0.05$). In juvenile

idiopathic arthritis associated uveitis patients (JIAU), Calprotectinlevels correlated with age and age at onset of uveitis. A longitudinal analysis in JIAU patients showed a correlation of serum Calprotectinlevels with uveitis activity (P = 0.03) (70).

Some studies have also analyzed if Calprotectin could be used to prognosticate response to certain drugs such as methotrexate and biological agents in patients with juvenile idiopathic arthritis. Moncrieffe at al (71) measured Calprotectin levels in a subgroup of patients with juvenile idiopathic arthritis prior to starting methotrexate therapy and demonstrated that patients with baseline Calprotectin levels greater than 3000 ng/ml were more likely to respond to methotrexate with an odds ratio of 16.07 and hence concluded that high levels of baseline serum Calprotectin have prognostic value in predicting a subgroup of patients whose arthritis will improve on methotrexate (71).

Anink et al tested the predictive value of serumCalprotectin levels in patients on treatment with tumour necrosis factor blocking agents (TNF blockers) to identify responders to treatment and also to see if it could predictrelapse after discontinuation of therapy. They found thatbaseline Calprotectinlevels were higher in responders (median Calprotectin level of 1466 ng/ml) compared to non-responders (median Calprotectin of 812 ng/ml, $p < 0.001$). Calprotectin levels decreased after commencement of treatment only in responders ($p < 0.001$). Calprotectin levels ($p = 0.031$, median 1025 ng/ml) were found to be higher in patients who flared within 6 months after discontinuation of treatment compared to patients with stable remission (median of 505 ng/ml) and hence concluded that higher levels of baseline Calprotectin was associated with good response

to anti-TNF treatment, whereas elevated Calprotectin levels at discontinuation of Etanercept was associated with higher chances to flare(72).

Thus, there are many studies from around the world regarding the various aspects of Calprotectin in multiple diseases and especially in JIA. But, studies analyzing Calprotectin as a marker of disease activity in Indian children are very few and hence we undertook the below mentioned study.

## AIM

To assess the usefulness of serum Calprotectin level as a marker of

disease activity in children with Juvenile Idiopathic arthritis

## OBJECTIVES

**Primary Objective:** To assess the usefulness of serum Calprotectin levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis.

**Secondary Objective:** To prove that serum Calprotectin levels is a better predictor of disease activity in children with JIA compared to ESR and CRP which are the present laboratory parameters used for assessment of disease activity.

# MATERIALS AND METHODOLOGY

## Study Design

Cross sectional study

## Study Period

Children were recruited over a period of one and half years from April 2016 to September 2017

## Study setting

Participants of this study were recruited from the Paediatric Rheumatology clinic of Christian Medical College, Vellore, India. The Paediatric Rheumatology outpatient department (OPD) functions on 3 week days (Wednesdays, Fridays and Saturdays) a week. The OPD serves on an average of about 60-90 patients every week.

## Participants

## Inclusion Criteria

Children diagnosed to have with JIA below the age of 16 years who fulfilled the International League of Associations for Rheumatology (ILAR) classification criteria for juvenile idiopathic arthritis (1).

**Exclusion criteria**

A) Children whose parents/local guardians did not give consent for recruitment into the study were excluded

B) Children with Inflammatory bowel disease, Kawasaki disease, Systemic Lupus Erythematosus and cystic fibrosis will be excluded as they can also have high Calprotectin levels.

**Data Collection and methodology:**

Children with Juvenile Idiopathic arthritis who presented serially to the pediatric rheumatology OPD in the above mentioned setting and time period and also fulfilled the inclusion criteria were recruited into the study. Clinical and relevant demographic data will be obtained from the participants directly into clinical proforma which contained details such as age, sex, disease duration, disease subtype, disease activity and laboratory parameters such as blood counts, ESR, CRP and Calprotectin levels .

Blood samples were collected for complete blood count, ESR and CRP which are the laboratory parameters being currently sent as standard of care for all patients with Juvenile Idiopathic Arthritis. In addition, blood for serum Calprotectin levels were also withdrawn by venipuncture method after obtaining consent from the parents for children below 12 years of age and assent from children above 12 years of age. Blood counts, ESR and CRP values were analysed on the same day. However, samples for Calprotectin analysis was stored in the department of clinical

microbiology in a temperature of -20 degree Celsius and all Calprotectin samples were analysed over a period of 2 days at the end of study period after recruitment of all the study patients. The laboratory values of the participants were obtained from the hospital patient information system.

Patients were divided into active and inactive group based on disease activity at the end of the study. The laboratory personnel analyzing the Calprotectin level were blinded about the disease activity status of the patients.

**Laboratory analysis:**

**Complete blood count:** Sample was sent in an EDTA tube and sent to the clinical pathology laboratory. Blood was processed in the Beckwith coulter machine and the validated results were considered.ESR was evaluated according to the Westergren method (cut-off 20mm/h).

**Serum CRP**: Measured by nephelometry assay; normal values ranged from 0.1 to 0.6 mg/dl.

**Serum Calprotectin:**

Serum Calprotectin levels were measured by sandwich-enzyme linked immune sorbent assay technology (ELISA) by using a "Human Calprotectin ELISA Kit" produced by Wuhan Fine Biological Technology Company limited. .

**Principle of the Assay:**

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti-Calprotectin antibody was pre-coated onto 96-well plates. And the biotin conjugated anti-Calprotectin antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the Calprotectin amount of sample captured in plate. The O.D. absorbance was read at 450nm in a microplate reader, and then the concentration of Calprotectin could be calculated.

**Materials Required:**

1.Human Calprotectin ELISA Kit (Kit components: ELISA micro Plate, lyophilized standard, dilution buffer, biotin labeled antibody, antibody dilution buffer, HRP-streptavidin conjugate, SABC dilution buffer, TMB substrate, Stop dilution, wash buffer, plate sealer)

2. Microplate reader (wavelength: 450nm)

3. 37°C incubator

4. Automated plate washer

5. Precision single and multi-channel pipette and disposable tips

6. Clean tubes and Eppendorf tubes

7. Deionized or distilled water

**Washing and sample collection for assay:**

All wells were aspirated and plates were washed 3 times with 350ul wash buffer. After the final wash, plate was inverted, and plates were clapped on absorbent filter paper or other absorbent material. Washer was set for a soaking time of 1 minute.

Serum samples were allowed to clot for 2 hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1000×g. The supernatant was collected and assay was carried out immediately. Blood collection tubes were disposable, non-pyrogenic, and non-endotoxin

**Dilution Factor:** The dilution factor used for this assay was 1:20

**Reagent Preparation and storage:**

All reagents were brought to room temperature before use.

**1, Wash Buffer:**

30mL of Concentrated Wash Buffer was diluted into 750 mL of Wash Buffer with deionized or distilled water. Unused solution was put back at 4°C. The solution was cooled to room temperature before use.

**2, Standard:**

1). 1000ng/ml of standard solution: 1 ml of Sample / Standard dilution buffer was added into one Standard tube, and the tube was kept at room temperature for 10 min and mixed thoroughly.

2).500ng/ml→15.63ng/ml of standard solutions: Label 6 Eppendorf tubes with500ng/ml, 250ng/ml, 125ng/ml, 62.5ng/ml, 31.25ng/ml, 15.63ng/ml, respectively. 0.3 ml of the Sample / Standard dilution buffer was added into each tube. 0.3 ml of the above1000ng/ml standard solution was added into 1st tube and mixed thoroughly. 0.3 ml from 1st tube to 2nd tube was added and mixed thoroughly. Transfer 0.3 ml from 2nd tube to 3rd tube and mixed thoroughly, and so on.

**3, Preparation of Biotin-detection Antibody working solution**

This was prepared within 1 hour before the experiment.

1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Dilute the Biotin-detection antibody with Antibody dilution buffer at 1:100 and mix thoroughly.(i.e. Add 1 μl of Biotin-detection antibody into 99 μl of Antibody dilution buffer.)

**4, Preparation of HRP-Streptavidin Conjugate (SABC) working solution:**

This was prepared within 30min before the experiment.

1) Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells. (Allow 0.1-0.2 ml more than the total volume)

2) Dilute the SABC with SABC dilution buffer at 1:100 and mix thoroughly. (i.e. Add 1 μl of SABC into 99 μl of SABC dilution buffer.)

**Assay Procedure**

Before adding to wells, the SABC working solution and TMB substrate was euilibrated for at least 30 min at room temperature (37 °C). When diluting samples and reagents, they were mixed completely and evenly and a standard curve was plotted for each test.

1. Standard, test sample and control (zero) wells were set on the pre-coated plate respectively, and then, their positions were recorded. It is recommend to measure each standard and sample in duplicate**.** Plates were washed 2 times before adding standard, sample and control (zero) wells!

2. Aliquot 0.1ml of 1000ng/ml, 500ng/ml, 250ng/ml, 125ng/ml, 62.5ng/ml, 31.25ng/ml, 15.63ng/ml, standard solutions into the standard wells.

3. 0.1 ml of Sample / Standard dilution buffer was added into the control (zero) well.

4. 0.1 ml of properly diluted serum sample was added into test sample wells.

5. The plate was sealed with a cover and incubated at 37 °C for 90 min.

6. The cover was removed and the plate content was discarded, the plate was clapped on the absorbent filter papers or other absorbent material.

7. 0.1 ml of Biotin-detection antibody working solution was added into the above wells (standard, test sample & zero wells). The solution at the bottom of each well was added without touching the side wall.

8. The plate was sealed with a cover and incubated at 37°C for 60 min.

9. The cover was removed and plate was washed 3 times with Wash buffer.

10. 0.1 ml of SABC working solution was added into each well, the plate was covered and incubated at 37°Cfor 30 min.

11. Remove the cover and wash plate 5 times with Wash buffer, and each time let the wash buffer stay in the wells for 1-2 min.

12. 90 µl of TMB substrate was added into each well, the plate was covered and incubated at 37°Cin dark within 15-30 min. And the shades of blue can be seen in the first 3-4 wells (with most concentrated Calprotectin standard solutions), the other wells show no obvious color.

13. Add 50 µl of Stop solution into each well and mix thoroughly. The color changes into yellow immediately.

14. Read the O.D. absorbance at 450 nm in a microplate reader immediately after adding the stop solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Calprotectin concentration of the samples can be interpolated from the standard curve by using professional software curve expert .Since the samples measured were diluted, the dilution factor was multiplied to the concentrations from interpolation to obtain the concentration before dilution.

**Summary**

1. Plate is washed 2 times before adding standard, sample and control (zero) wells!

2. 100μL standard or sample is added to each well for 90 minutes at 37°C. Aspirate and wash plate 2 times.

3. add 100μL Biotin-detection antibody working solution to each well for 60 minutes at 37°C

4. Aspirate and wash 3 times

5. Add 100μLSABCworking solution to each well. Incubate for 30 minutes at 37°C

6. Aspirate and wash 5 times

7. Add 90μLTMB substrate. Incubate 15 -30 minutes at 37°C

8. Add 50µL Stop Solution. Read at 450nm immediately

9. Calculation of results

## Statistical Methods:

Continuous variables such as ESR and CRP were described as mean with standard deviation or median and interquartile range (IQR), and categorical variables as frequencies with percentages. The diagnostic performance of Calprotectin level was assessed by plotting ROC for active and non-active cases. ROC was also plotted for diseased and healthy controls and the cut off with a good sensitivity and specificity was chosen.Spearman's rank correlation coefficient was applied to correlate Calprotectin with disease activity . The comparison of ESR. CRP and Calprotectin across active and inactive disease was done using Mann Whitney U test as the distribution of the variables was slightly skewed which was assessed using QQ plot. The sensitivity and specificity of ESR, CRP and Calprotectin were obtained for best cut off values using ROC curve. The area under ROC curves for ESR, CRP and Calprotectin levels was compared to find which among the three serves as a good marker of disease activity. ESR, CRP and Calprotectinwere correlated using Pearson's correlation or Spearman's correlation.

Box plots were presented for the distribution of continuous variables across active and inactive groups. P value < 0.05 was considered as statistical significance. SPSS 16 was used for analyzing the data.

## RESULTS

121 children were recruited during study period. Baseline demographics and blood investigations recorded were analysed. At the end of the study, Children with Juvenile Idiopathic Arthritis (JIA) were divided into two groups. Group I was JIA children with active disease (n=68) and Group 2 was JIA children with inactive disease (n=58) as assessed by Wallace criteria. We also estimated Calprotectin levels in 10 normal children.

**Table 1: Demographic characteristics of all children (n=121)**

| Parameter | Number (%) (n =121) |
|---|---|
| Gender | Male 68 (56%)<br>Female 53 (44%) |
| Mean age at diagnosis (years) | 11.15 ± 3.75 |
| Diagnosis<br>• Oligoarticular JIA<br>• Polyarticular RF pos<br>• Polyarticular RF neg<br>• Enthesitis related arthritis<br>• SOJIA | <br>• 8 (7 %)<br>• 4 (3%)<br>• 37 (31%)<br>• 20 (16%)<br>• 52 (43%) |
| Disease duration (years) | 3.86 ± 2.64 ( 1 – 14) |
| Disease status<br>• Active<br>• Remission on drugs<br>• Remission off drugs | <br>• 63 (52%)<br>• 57 (47%)<br>• 1 (1%) |

**Table 2: Baseline Characteristics of Active and Inactive disease**

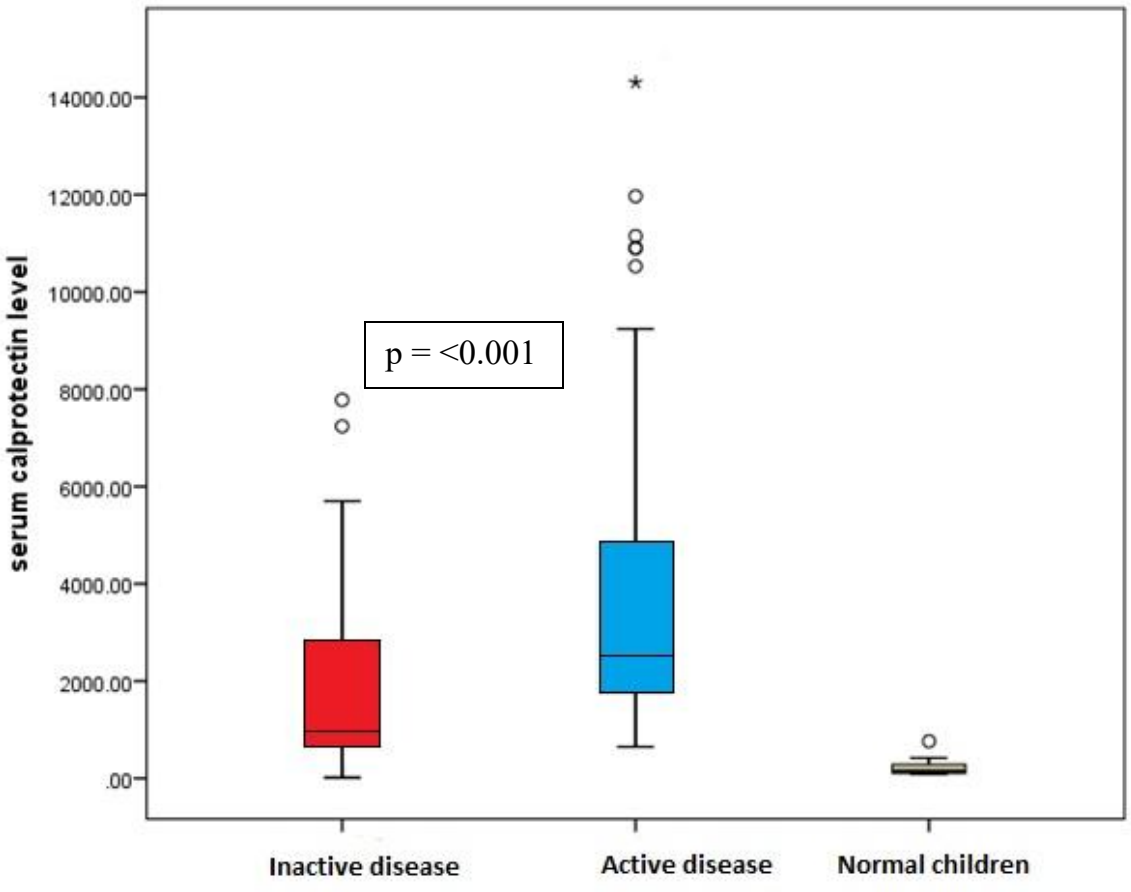
| Baseline characteristics | JIA (n=121) | |
|---|---|---|
| Disease status | Active disease (n= 63) | Inactive disease (n= 58) |
| Age (years) | 11.30±3.88 | 11.00±3.60 |
| **JIA SUBTYPE:** | | |
| Oligoarticular | 3 | 5 |
| RF + Polyarticular JIA | 1 | 3 |
| RF – Polyarticular JIA | 13 | 24 |
| Systemic JIA | 37 | 15 |
| ERA | 9 | 11 |
| Hemoglobin gm/dl | 10.02±1.46 | 12.04±1.77 |
| Platelet (cu mm) | 453819 ± 146032 | 324034 ±114691 |
| Total Count | 19782 ± 29612 | 9191 ± 2836 |
| ESR (mm/hour) | 46.71±17.14 | 14.24±8.07 |
| CRP (mg/dl) | 100.65±69.83 | 3.07±0.26 |

**Table 3: Mean and standard deviation of Calprotectin in various groups:**

| Calprotectin (ng/ml) | Normal children (n=10) | Active Disease (n=63) | Remission (n=58) | P value |
|---|---|---|---|---|
| Mean (ng/ml) | 233 ±220 | 3954 ± 3220 | 1899± 1765 | < 0.001 |
| Range(ng/ml) | 86-764 | 651- 14310 | 160 – 7780 | |

Mean value of Calprotectin was much higher in children with active disease as compared to children with inactive disease. Children with active disease had Calprotectin levels which were 2 fold higher than those with inactive disease. Children with inactive disease had Calprotectin levels which were in turn, 8 times higher than children who were normal healthy controls. Calprotectin values in all groups (active disease, inactive disease and healthy controls) were statistically significant ($p = <0.001$).

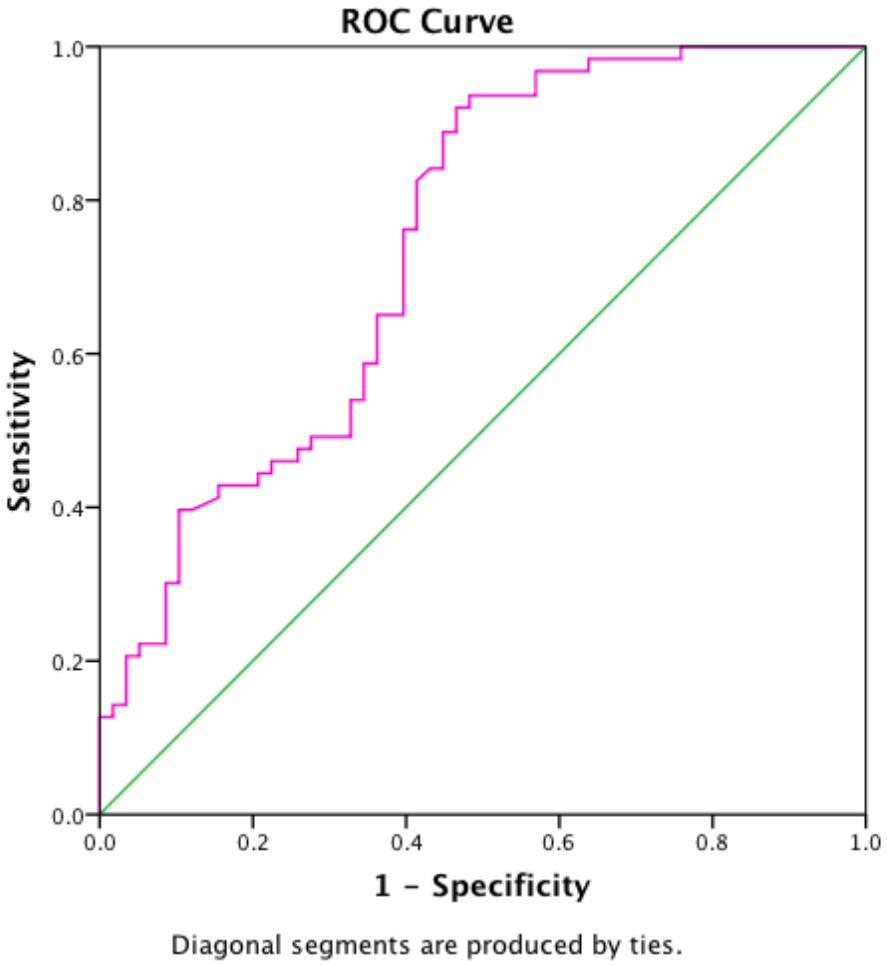
**Fig 1: Box Plot ofCalprotectin Values in children with active disease, remission and in normal children**




Horizontal lines show the medians; boxes show the interquartile ranges; vertical lines show the high and low values.

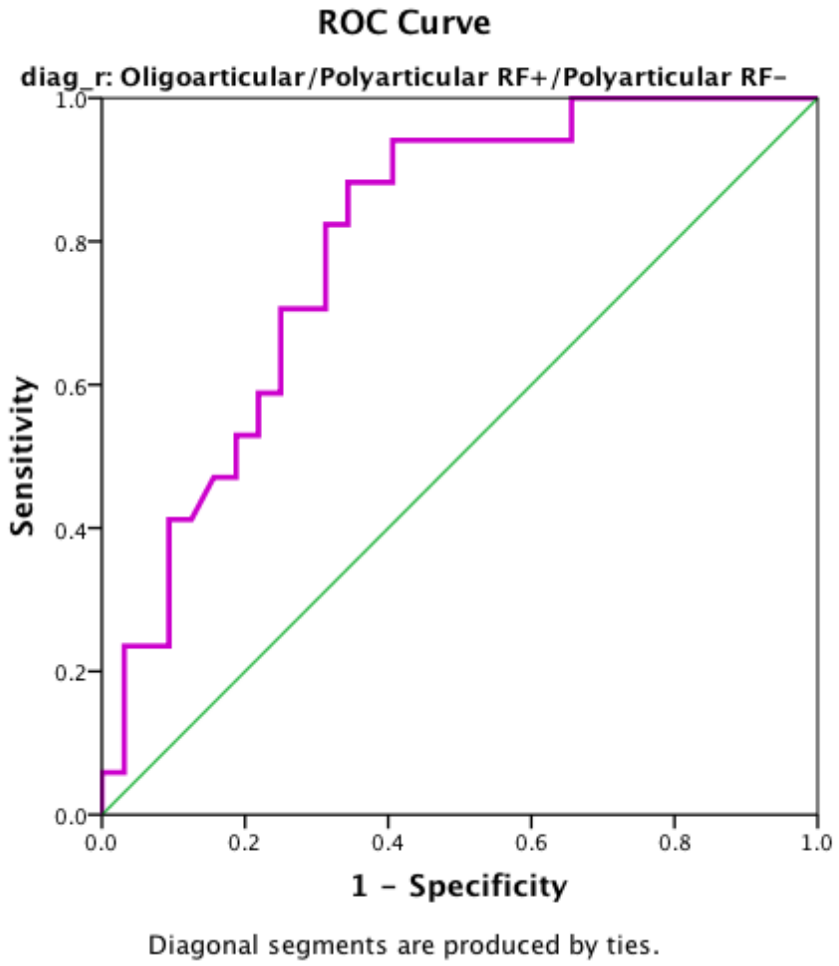
Mean Calprotectin levels in children with active disease was 3954 ng/ml and those in inactive disease was 1899ng/ml. Mean calprotectin levels in normal healthy children was only 233ng/ml. The above box plot clearly indicates that Calprotectin levels were much higher in children with active disease when compared to children with inactive disease and healthy children.

**Fig 2: ROC curve analysis showing the diagnostic performance of Calprotectin for discriminating patients with active JIA from patients with inactive disease:**



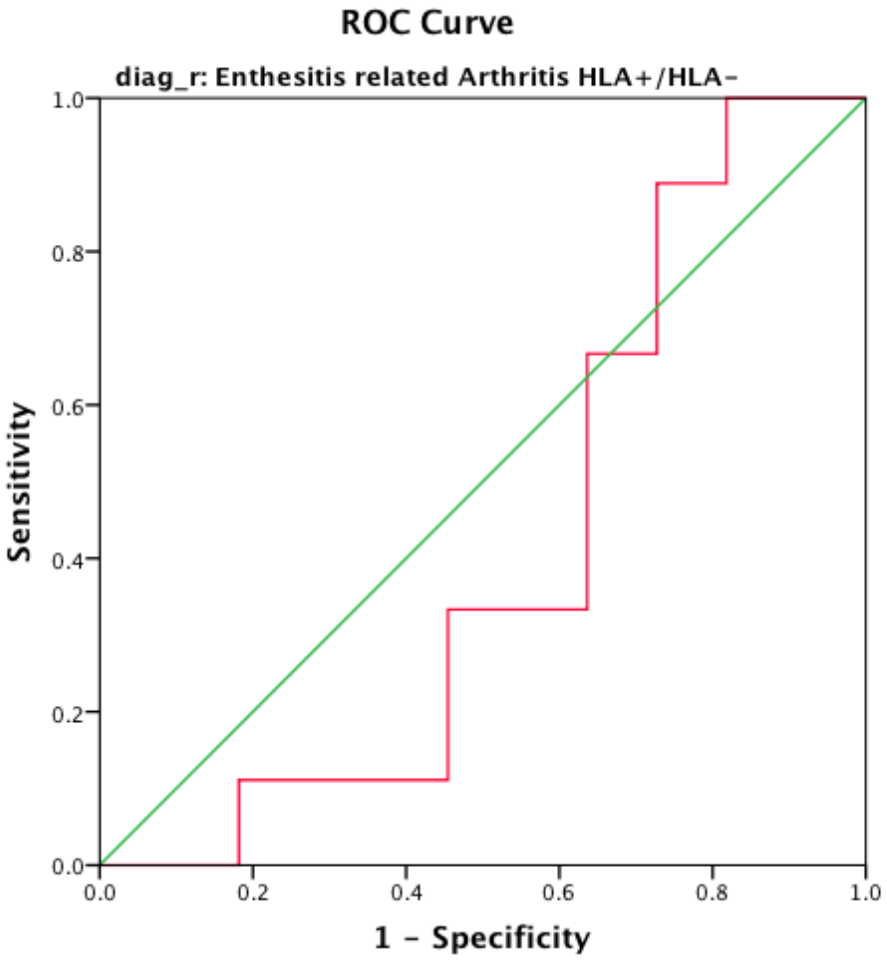

The Area under the curve for Calprotectin was 0.744 . For a value of 1760 ng/ml, Calprotectin had a sensitivity of 77% and specificity of 61% .

**Fig 3: ROC curve of Calprotectin in children with oligoarticular and polyarticular JIA**



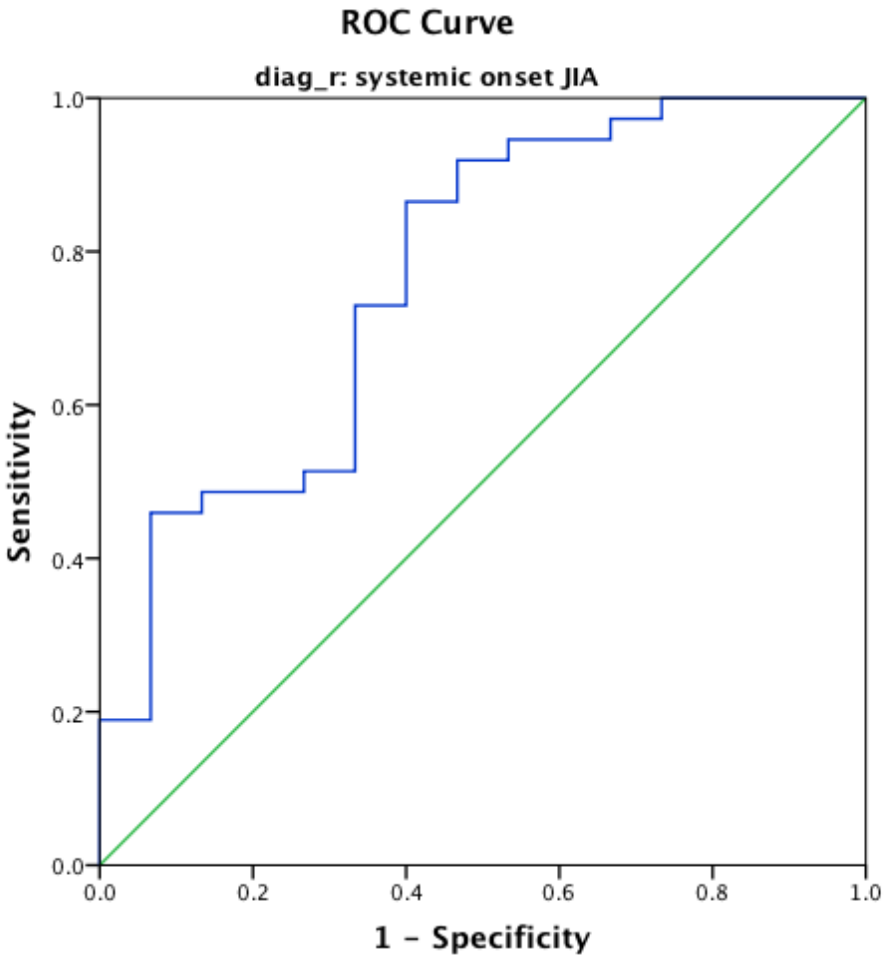

Area under the curve for Calprotectin in children with Oligoarticular and polyarticular JIA( RF +ve and RF –ve ) was 0.797 . In children with the above mentioned subtype, a Calprotectin value of 1468ng/ml had a sensitivity of 82% and specificity of 69%

**Fig4: ROC curve of Calprotectin in children with Enthesitis related arthritis**



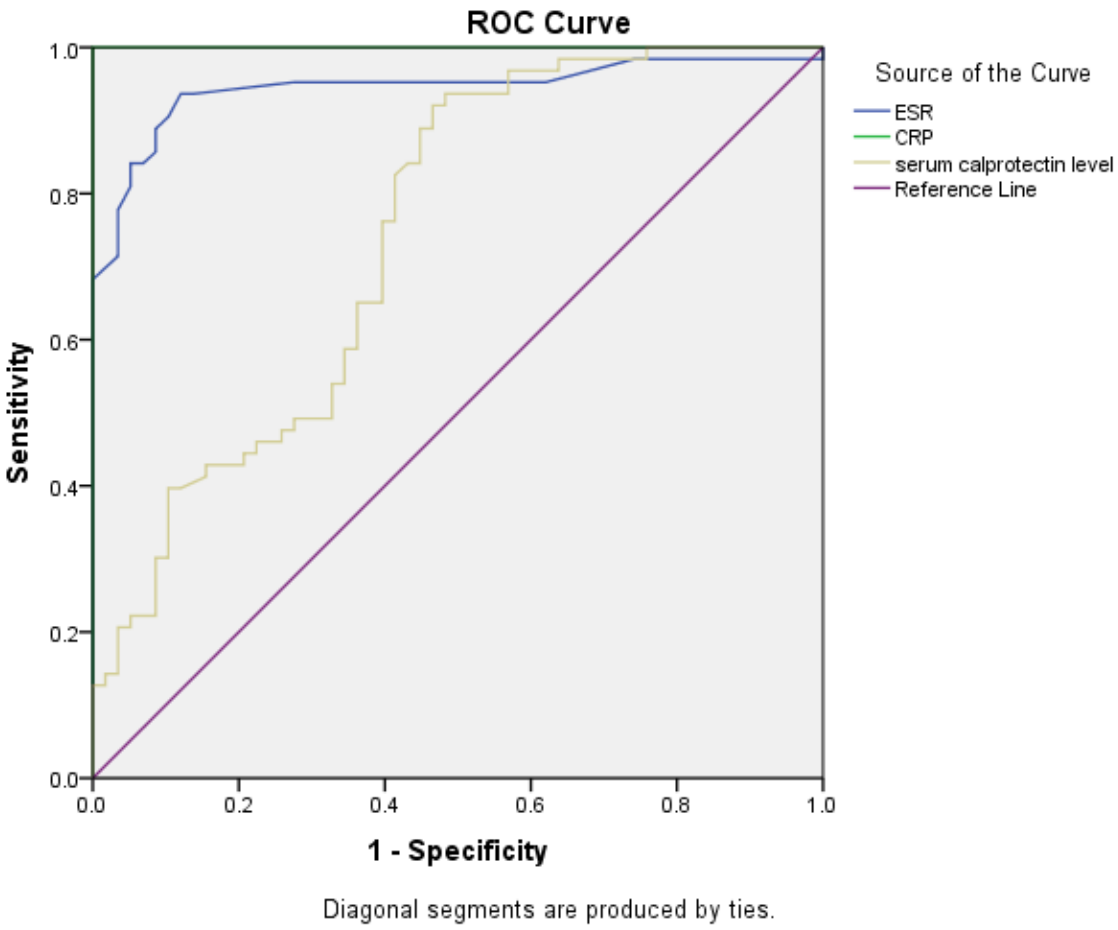

Area under the curve for Calprotectin in children with Enthesitis Related Arthritis ( HLA +ve and HLA –ve ) was only 0.414 . In children with the above mentioned subtype, a Calprotectin value of 2141ng/ml had a sensitivity of 77% and specificity of 28%

**Fig5: ROC curve of Calprotectin in children with Systemic onset JIA**



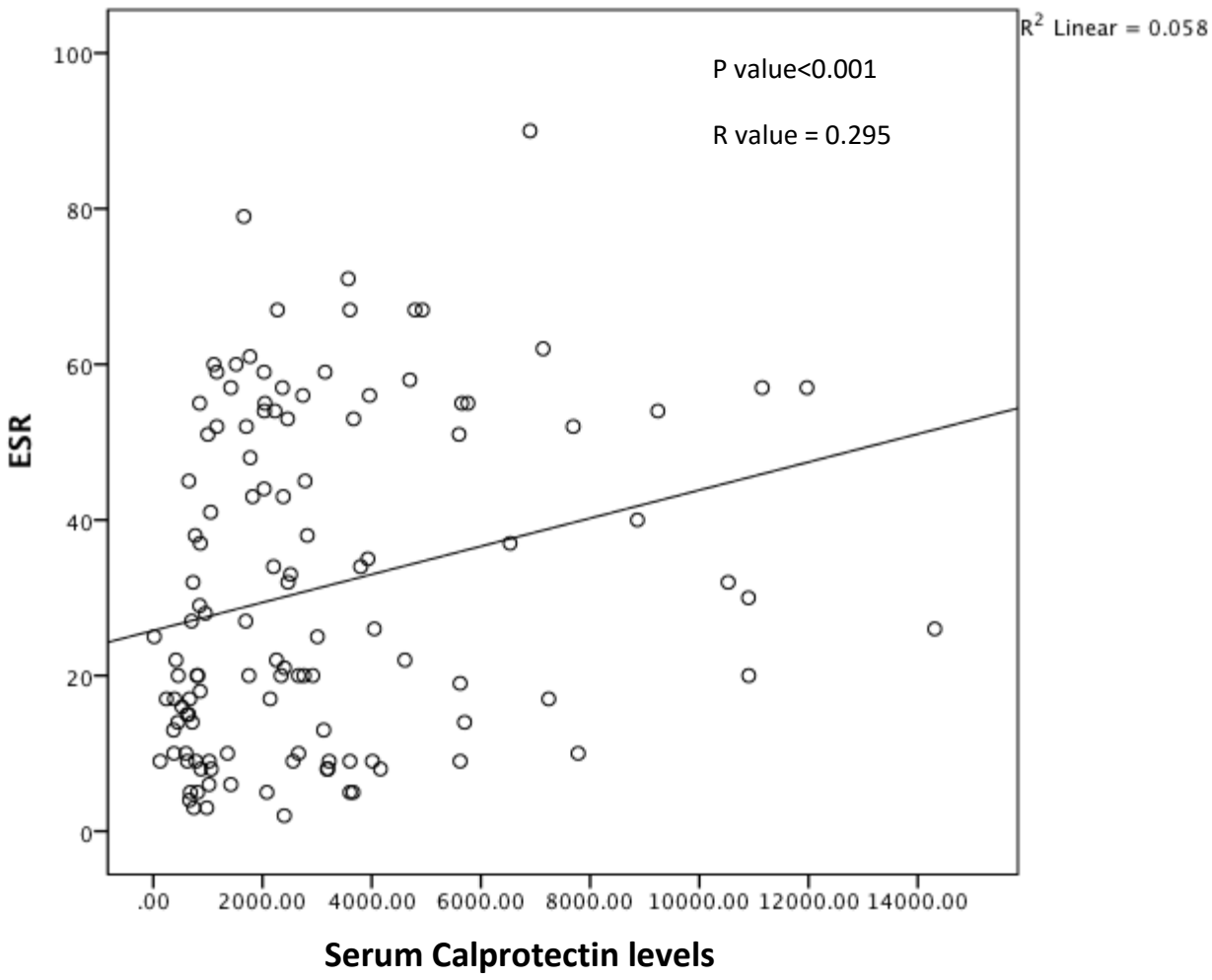

Area under the curve for Calprotectin in children with Systemic onset JIA was 0.768 . In children with the above mentioned subtype, a Calprotectin value of 1111ng/ml had a sensitivity of 86 % and specificity of 60 %

**Fig6: Comparison of ROC curve of ESR, CRP and Calprotectin:**



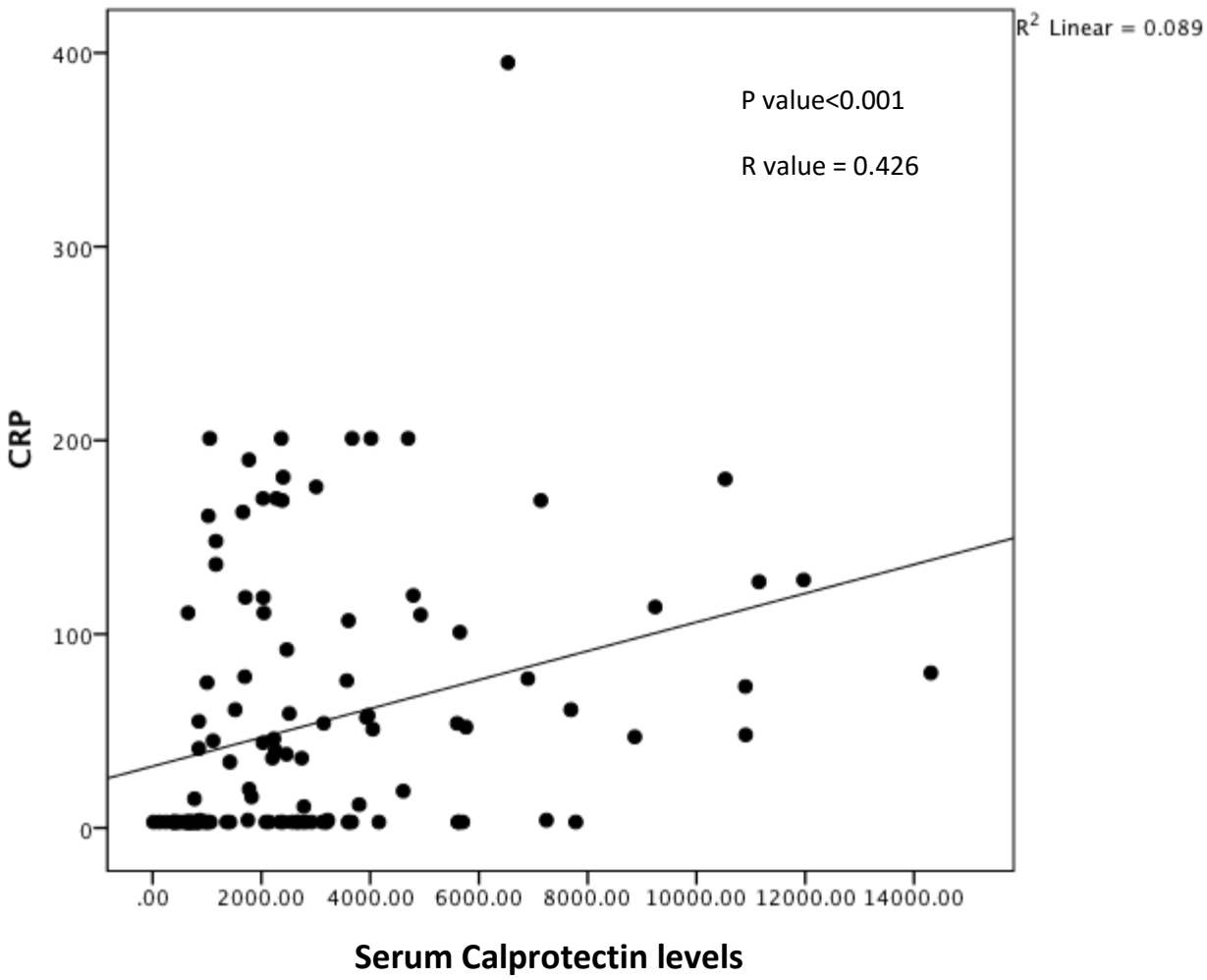

The area under the curve for CRP, ESR and Calprotectin were 1.0, 0.944 and 0.744 respectively. Hence, based on the ROC curve, it can be inferred that ESR and CRP are better markers of disease activity as compared to Calprotectin.

**Fig 7: Scatter plot of correlation between ESR and Calprotectin**




Based on the scatter plot, it can be inferred that there was only weak correlation between ESR and Calprotectin. r value was 0.295 and p value was <0.001

**Fig 8: Scatter plot of correlation between CRP and Calprotectin**




There was only a weak correlation between CRP and Calprotectin with an r value of 0.426 and p value<0.001

# DISCUSSION

As mentioned in the introduction, JIA is the most common chronic rheumatic disorder of childhood. Even though, clinical criteria for JIA to differentiate active and inactive disease are fairly well defined, there is lack of reliable laboratory criteria which can differentiate disease activity. Various biomarkers which can reliably predict disease activity are currently under research. Calprotectin is one such marker which is currently under extensive research. We undertook this study to assess the usefulness of Calprotectin as marker of disease activity in Indian children with juvenile idiopathic arthritis.

**Demographic Characteristics:**

We studied a total of 121 children with juvenile idiopathic arthritis. In our study, there was a slight preponderance of males who constituted 56% of the study population as compared to females who constituted 44% of study population. This was in consistence with the results published by Porkodi et al who studied clinical profile of JIA in children from South India and showed a male preponderance of 62%.(73). Singh et al studied the clinic immunological profile of children with JIA in North India (Chandigarh) and found a male preponderance of 64%.(74)

Male:female ratio in our study was 1.2:1. This resonated with similar ratios demonstrated by Saurenmann et al in their study of ethnicity as a risk factor in children with JIA. In

their study, they found that the male:female ratio in children with JIA of Asian descent was 1:1. (75)

The mean age at diagnosis in our study, taking into account all 121 children was 11.15 ± 3.75 years.

In our study, 52% of children had systemic onset JIA constituting the maximum number. Children with polyarticular, pauciarticular and enthesitis related arthritis constituted 41%, 8% and 20% of our study population respectively. In a study done by Viswanatha Kumar et al who studied 112 children with JIA, 9 % had systemic onset, 63% had polyarticular onset and 36 % had pauci-articular onset JIA(76). Similarly, Seth et al studied the clinic immunological profile of 361 children with JIA.The frequency of occurrence of systemic, pauciarticular and polyarticular disease in their study was 24%, 30% and 46% respectively(77). Although, it is difficult to provide an explanation for these differences, it may be related to the different genetic backgrounds of the populations under study. It is known that some HLA haplotypes are closely associated with certain subtypes of JIA. These differences might also have occurred because the studies done by Viswanatha Kumar et al and Seth et al did not take children with enthesitis related arthritis into consideration in their studies. Maximum number of children in our study had systemic onset JIA, this might have been because of the fact that, children with systemic onset JIA have more frequent follow up and hospital visits as part of our treatment protocol compared to children with other subtypes because of their disease severity.

In our study, the mean value of Calprotectin in children with active disease (all disease subtypes included) was 3954 ng/ml and that in inactive disease was 1899 ng/ml. This difference was statistically significant (p=<0.001). The mean value of Calprotectin levels in normal healthy controls was233 ng/ml. Thus, Calprotectin levels in patients with inactive disease were 8 times more than in normal healthy controls. Children with active disease had 2 fold increased levels of Calprotectin as compared to children with inactive disease. Badot et al(78) conducted a study in Belgium and measured Calprotectin levels in 95 children with Juvenile Idiopathic arthritis. The findings of their study were closely similar to that of ours. Badot et al found that patients with inactive JIA had a 4-fold increased level of serum Calprotectin (6.555 ng/mL) compared to healthy controls (1.737 ng/mL), while patients with active JIA had themselves a 2-fold increased level of serum Calprotectin (11.403 ng/mL) compared to patients with inactive disease.

The role of serum Calprotectin as a marker of disease activity in systemic onset juvenile idiopathic arthritis has been investigated in multiple studies. In our study, Calprotectin was found to be an excellent marker of disease activity in children with systemic onset Juvenile Idiopathic arthritis. The mean value of Calprotectin in children with active disease was 4415 ng/ml which was significantly higher than the value of Calprotectin in children with inactive disease who had a mean Calprotectin value of 1901ng/ml. Calprotectin mean value in active disease was 18 times more than the mean value obtained in normal healthy controls (233ng/ml). The median Calprotectin value in active and inactive disease groups in our study were 2740ng/ml and 1020 ng/ml respectively. .

Findings similar to ours were found in the study done by Frosch et al(52). In their study, serum Calprotectin levels during active disease in patients with systemic-onset JIA were 44-fold higher than those in healthy controls (mean 14,920 ng/ml versus 340 ng/ml). Patients with inactive disease had a mean value of 530ng/ml. Mean concentrations during active disease in patients with systemic-onset JIA were also significantly higher than those in patients with systemic infections (2,640 ± 720 ng/ml), acute lymphoblastic leukemia (650 ±280 ng/ml), acute myeloblastic leukemia (840 ±940 ng/ml), and NOMID (2,830 ±580 ng/ml) . However, such comparisons cannot be made in our study as we looked at Calprotectin values in children with juvenile idiopathic arthritis only and did not include children with other conditions as mentioned in Frosch's study.

A study done by Holzinger et al(66) also resonated the fact that Calprotectin levels are very high in children with active disease as compared to those in disease remission with a mean Calprotectin value of 12030ng/ml during active phase of illness as against mean of 864ng/ml during disease remission. Holzinger's study also proved that during clinical remission, serum Calprotectin levels greater than740 ng/ml predicted disease flares accurately with a sensitivity of 92% and specificity of 88%. However, this specific aspect of Calprotectin as marker to predict a disease flare during a period of clinical remission was not looked at in our study.

In our study, the area under the curve for Calprotectin in children with systemic onset JIA was 0.768. In children with systemic onset JIA, a Calprotectin value of 1111ng/ml had a

sensitivity of 86 % and specificity of 60 %. Shenoi et al(65) studied various biomarkers in children with systemic onset JIA and in their study,. Calprotectin levels were 8 times higher in children with active systemic onset JIA as against children with inactive disease. The area under the curve for Calprotectin in their study was .86 with 80% sensitivity and 80% specificity. The difference in the specificity and AUC values between our study and Shenoi's study might be because of differences in sample size between the 2 study groups. We had 52 children with systemic onset JIA in our study as against only 20 children in Shenoi's study.

In our study, Calprotectin levels correlated well with disease activity in children with oligoarticular juvenile idiopathic arthritis with mean value of 2738 ng/ml in children with active disease compared to a mean value of 428 ng/ml in children with inactive disease. The mean value of Calprotectin was 6 times more elevated in children with active disease as compared to those with inactive disease in oligoarticular JIA subgroup in our study. These findings were consistent with similar studies done elsewhere. Frosch et al (68)measured Calprotectin levels in children with oligoarticular JIA and found that Calprotectin levels were up to 5 times higher in serum samples from patients with active disease (mean of 2,170ng/ml) compared with those in remission (mean of 405 ng/ml) . Those in remission showed levels that were only slightly elevated over the normal range (mean of 360 ng/ml). They also found that Calprotectin levels were much higher in synovial fluid as compared to serum and that serum levels of Calprotectin decreased after

treatment with intraarticular triamcinolone injections in patients who responded to therapy. However, such comparisons could not be made in our study as we did not look at these specific parameters such as synovial fluid Calprotectin levels or changes in Calprotectin levels with regard to drug therapy.

In our study Calprotectin was not useful as a good marker of disease activity in children with Enthesitis related arthritis. The median Calprotectinlevel in active disease was 2466 ng/ml and median Calprotectin level in inactive disease was 2710 ng/ml. However, in contrast, in a study done by Rahman et al in India(69), who measured Calprotectin levels in 69 patients with enthesitis related arthritis,Patients with active disease (11 669.5 ng/ml) had higher levels Calprotectin as compared with inactive disease (4421.8 ng/ml, $P<0.0001$). There is no logical explanation as to why median levels of Calprotectin were lower in the active disease group as compared to inactive disease group in our study.

In our study, apart from analyzing the values of Calprotectin with regard to disease activity, we also compared Calprotectin with ESR and CRP which are the current biomarkers being used for assessment of disease activity. In our study, there was weak correlation between ESR and Calprotectin with r value of 0.295 and p value was <0.001. Similarly, weak correlation was found between CRP and Calprotectin with r value of 0.426 and p value <0.001. These findings of ours were consistent with a study done by Bojka et al(62) where weak correlation was found between ESR and Calprotectin with a

*p* value of 0.000079 and r value of 0.450. Like in our study, only weak correlation existed between CRP and Calprotectin with a *p* value of 0.000058 and r value of 0.480.

Thus, our study proves the usefulness of serum Calprotectin levels as a good marker of disease activity in children with Juvenile Idiopathic arthritis except in the enthesitis related arthritis subgroup. However, we could not prove that Calprotectin was better than ESR and CRP as a disease activity biomarker as claimed by other studies from various parts of the world.

## LIMITATIONS

There were a few limitations to our study:

1. Firstly, considering the fact that the objective of the study was to assess the usefulness of Calprotectinas a biomarker of disease activity in children with JIA, collection of serial samples in same children would have been ideal. Comparisons based on sequential sampling may have provided additional information on the accuracy of these markers.

2. Secondly, since we studied Calprotectin levels in JIA as a whole, there was unequal distribution of patients in various disease subsets. This might have had a few implications on the results of this study.

3. In our study, all serum samples for measurement of Calprotectin were analysed on a single day, and hence, samples had been stored in Microbiology lab for periods ranging from 1 month to 1 year. The implication of storage and its effect on estimated values of Calprotectin during analysis remains unknown.

## SUMMARY OF RESULTS

- 121 children with JIA were recruited into the study, 63 had active disease and 58 had inactive disease.
- Systemic onset JIA constituted 42% of the study population and was the predominant disease subtype.
- Mean Calprotectin value in active disease (3954ng/ml) was 2 fold higher than those with inactive disease (1899ng/ml) (p value <0.001) and 16 times higher than children who were normal healthy controls(mean of 233ng/ml).
- For a cut off value of 1760 ng/ml, Calprotectin had a sensitivity of 77% and specificity of 61% for assessment of disease activity in JIA.
- Calprotectin was best suitable as a disease marker for systemic onset subtype of JIA where it had an area under the curve of 0.768 and for a cut off value of 1111ng/ml, had a sensitivity of 86 % and specificity of 60 %.
- However, Calprotectin correlated very poorly with ESR and CRP with r values of 0.295 and 0.426 respectively..
- Hence, from our study, we can conclude that Calprotectin is a good marker of disease activity in children with JIA. However, it was not superior to ESR or CRP.

## CONCLUSION

To conclude, our data suggest that Calprotectin levels are increased in the plasma of JIA children and plasma Calprotectin correlates with disease activity. However, it was not found to be superior to ESR and CRP. But, it seems logical to suspect that Calprotectin will be a better marker for follow up of disease activity in patients receiving biological therapy as these drugs act against cytokines such as TNF-α, interleukin-1 and interleukin 16 which will reduce the circulating cytokines and thus levels of acute phase reactants such as ESR and CRP. Thus, we need more studies in Indian population which involve sequential sampling of the same study population in their various phases of disease activity and also look at the effect of biological agents and other medications on the levels of these biomarkers which will enable us to precisely understand the accuracy of these biomarkers. A prospective study with a longer follow-up will help establish the role of Calprotectin in monitoring disease activity and predicting flares.

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# ANNEXURES

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

Dr. B.J. Prabhantham, M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

Dr. Alfred Job Daniel, D Ortho MS Ortho DNB Ortho.
Chairperson, Research Committee & Principal

Dr. Biju George, MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

June 02, 2016

Dr. Anish Sam George,
PG Registrar,
Department of Child Health II
Christian Medical College,
Vellore 632 004.

Sub: **Fluid Research Grant NEW PROPOSAL:**
Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis.

Dr. Anish Sam George (Employment Number: 33430), Post Graduate Registrar, Child Health unit 2, Dr. Sathish Kumar.T (Employment Number: 20174), Child Health unit 2, Dr. John Antony Jude Prakash (Emp. No. 14982), Clinical Microbiology.

Ref: IRB Min No: 9977 [DIAGNO] dated 02.03.2016

Dear Dr. Anish Sam George,

I enclose the following documents:-

1. Institutional Review Board approval 2. Agreement

Could you please sign the agreement and send it to Dr. Biju George, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Biju George
Secretary (Ethics Committee)

Dr. BIJU GEORGE
MBBS., MD., DM.
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002.

OFFICE OF RESEARCH
INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

**B.J. Prashantham,** M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

**Dr. Alfred Job Daniel,** D Ortho MS Ortho DNB Ortho
Chairperson, Research Committee & Principal

**Dr. Biju George,** MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
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June 02, 2016

Dr. Anish Sam George,
PG Registrar,
Department of Child Health II
Christian Medical College,
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Sub: **Fluid Research Grant NEW PROPOSAL:**
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Dr. Anish Sam George (Employment Number: 33430), Post Graduate Registrar , Child Health unit 2, Dr. Sathish Kumar.T (Employment Number: 20174), Child Health unit 2, Dr. John Antony Jude Prakash (Emp. No. 14982), Clinical Microbiology.

Ref: IRB Min No: 9977 [DIAGNO] dated 02.03.2016

Dear Dr. Anish Sam George,

The Institutional Review Board (Blue, Research and Ethics Committee) of the Christian Medical College, Vellore, reviewed and discussed your project titled "Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis" on March 02nd 2016.

The Committee reviewed the following documents:

1. IRB Application format
2. Patient Information Sheet and Informed Consent Form (English, Tamil, Hindi)
3. Cvs of Drs. John Jude, Anish, Sathish.
4. Proforma.
5. No. of documents 1 – 4

The following Institutional Review Board (Blue, Research & Ethics Committee) members were present at the meeting held on March 02nd 2016 in the CREST/SACN Conference Room, Christian Medical College, Bagayam, Vellore 632002.

**B.J. Prashantham,** M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

**Dr. Alfred Job Daniel,** D Ortho MS Ortho DNB Ortho
Chairperson, Research Committee & Principal

**Dr. Biju George,** MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

| Name | Qualification | Designation | Affiliation |
|---|---|---|---|
| Dr. Biju George | MBBS, MD, DM | Professor, Haematology, Research), Additional Vice Principal , Deputy Chairperson (Research Committee), Member Secretary (Ethics Committee), IRB, CMC,Vellore | Internal, Clinician |
| Dr. Anuradha Rose | MBBS, MD, MHSC (Bioethics) | Associate Professor, Community Health, CMC, Vellore | Internal, Clinician |
| Dr. Jayaprakash Muliyil | BSc, MBBS, MD, MPH, Dr PH (Epid), DMHC | Retired Professor, Vellore | External, Scientist &Epidemiologist |
| Rev. Joseph Devaraj | BSc, BD | Chaplaincy Department, CMC, Vellore | Internal, Social Scientist |
| Ms. Grace Rebekha | M.Sc., (Biostatistics) | Lecturer, Biostatistics, CMC, Vellore | Internal, Statistician |
| Dr. Visalakshi. J | MPH, PhD | Lecturer, Biostatistics, CMC, Vellore | Internal, Statistician |
| Mrs. Sheela Durai | MSc Nursing | Professor, Medical Surgical Nursing, CMC, Vellore | Internal, Nurse |
| Dr. Simon Pavamani | MBBS, MD | Professor, Radiotherapy, CMC, Vellore | Internal, Clinician |
| Mrs. Pattabiraman | BSc, DSSA | Social Worker, Vellore | External, Lay Person |
| Dr. B. J. Prashantham | MA(Counseling Psychol MA(Theology). Dr. Min(Clinical Counselling) | Chairperson, Ethics Committee, IRB. Director, Christian Counseling Cent Vellore | External, Social Scientist |
| Dr. Rajesh Kannangai | MD, PhD. | Professor, Clinical Virology, CMC, Vellore | Internal, Clinician |

# OFFICE OF RESEARCH
# INSTITUTIONAL REVIEW BOARD (IRB)
# CHRISTIAN MEDICAL COLLEGE, VELLORE, INDIA

**B.J. Prashantham,** M.A., M.A., Dr. Min (Clinical)
Director, Christian Counseling Center,
Chairperson, Ethics Committee.

**Dr. Alfred Job Daniel,** D Ortho MS Ortho DNB Ortho
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**Dr. Biju George,** MBBS., MD., DM
Deputy Chairperson,
Secretary, Ethics Committee, IRB
Additional Vice-Principal (Research)

| | | | |
|---|---|---|---|
| Mrs. Emily Daniel | MSc Nursing | Professor, Medical Surgical Nursing, CMC, Vellore | Internal, Nurse |
| Dr. Sathish | MBBS, MD, DCH | Professor, Child Health, CMC, Vellore | Internal, Clinician |
| Dr. Rekha Pai | MSc, P.hd | Internal Basic Scientist, Int Basic Scientist, CMC, Vellore | External, Legal Expert |
| Dr. Anand Zachariah | MBBS, PhD | Professor, Medicine, CMC, Vellore | Internal, Clinician |

We approve the project to be conducted as presented.

Kindly provide the total number of patients enrolled in your study and the total number of withdrawals for the study entitled: "Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis" on a monthly basis. Please send copies of this to the Research Office (research@cmcvellore.ac.in).

*Fluid Grant Allocation:*

*A sum of 98,750/- INR (Rupees One Lakh Only) will be granted for 2 years. 50,000/- INR (Rupees Fifty Thousand only) will be granted for 12 months as an Ist Installment. The rest of the 48,750/- INR (Rupees Forty Eight thousand seven hundred and fifty only) each will be released at the end of the first year as 2 nd Installment.*

Yours sincerely

Dr. Biju George
Secretary (Ethics Committee)
Institutional Review Board

**Dr. BIJU GEORGE**
MBBS., MD., DM
SECRETARY - (ETHICS COMMITTEE)
Institutional Review Board,
Christian Medical College, Vellore - 632 002

**Christian Medical College, Vellore**

**Department of Pediatrics**

## Study Title: Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis

**Study Information sheet**

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You are being requested to participate in a study to measure Calprotectin levels in blood for the estimation of disease activity in children with Juvenile Idiopathic Arthritis

**What is Juvenile Idiopathic arthritis (JIA)?**

Juvenile Idiopathic arthritis is one of the common rheumatic diseases affecting children less than 16yrs of age. It is a systemic inflammatory disorder involving synovial joints. Characteristic features of JIA include painful swelling of the joints with painful restriction of the movements, lasting for more than six weeks of duration. JIA is further classified into different subtypes based on the number of joints involved. JIA is treated with Non-steroidal anti-inflammatory drugs, Disease modifying antirheumatic drugs, biological agents or steroids based on the disease status.

**What is Serum Calprotectin? Why is it important to estimate its level?**

Inflammation is a process in which white blood cells and chemicals help protect us from infection and foreign substances such as bacteria and viruses. In some diseases, however, the body's defence system (immune system) triggers an inflammatory response when there are no foreign substances to fight off which is called autoimmune diseases, where the body's normally protective immune system causes damage to its own tissues

JIA is an autoimmune disease affecting a large number of children. Even today, there are no reliable laboratory tests for assessment of disease activity in JIA. Hence, there are a number tests undergoing research for assessment of disease activity in JIA. . Serum Calprotectin is one such test.

Calprotectin is a protein found in blood and is released by leucocytes (white blood cells) during periods of inflammation as occurs in JIA. Western studies have proved Calprotectin levels are increased in children with JIA. But studies in Indian Children are lacking. Our aim is to prove Calprotectin levels in blood will aid as a marker for assessment of disease activity in children with JIA. If proved by our study, it will help in the diagnosis and management of children with JIA in the future.

**If you take part what will you have to do?**

If you agree to participate in this study, your child will be enrolled into the study. Blood samples will be taken for CBC, ESR, CRP and serum Calprotectin by simple venipuncture method. There are nil adverse effects for this simple procedure except minimal pain at the time of blood draw.

**Can you withdraw from this study?**

Your participation in this study is entirely voluntary and you are also free to decide to withdraw permission to participate in this study. If you do so, this will not affect your child's usual treatment at this hospital in any way.

**What will happen if you develop any study related injury?**

Study related injury is very unlikely as this study involves blood sampling by simple venipuncture method which is minimally invasive.

**Will you have to pay for the treatment and investigations?**

Yes. You have to pay for Complete Blood Count, ESR and CRP which are routine tests being currently done at CMC for your child's assessment and treatment. Serum Calprotectin levels will be done as part of this study and you do not have to pay for this test.

**What happens after the study is over?**

Once test is performed, your child can continue the treatment as advised. Being enrolled in this study will not affect your child's treatment in any way..

**Will your personal details be kept confidential?**

The results of this study will be published in a medical journal in the future but your child will not be identified by name in any publication or presentation of results. However, your medical notes may be reviewed by people associated with the study, without your additional permission.

**Who to contact in case of any further study related doubts?**

If you have any further clarifications, kindly contact

Dr. Anish Sam George,
PG Registrar,
Department Of Child Health,
CMC, Vellore
Mob: 9840868253
E-mail: dr.anishsam@gmail.com

**Christian Medical College, Vellore**

**Department of Pediatrics**

## CONSENT TO TAKE PART IN THE STUDY

**Study Title: Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis**.

**Study Number:**
**Participant's name:**
**Date of Birth / Age (in years)**:

I_____
_____, father/mother/guardian of _____

(Please tick boxes)
Declare that I have read the information sheet provided to me regarding this study and have clarified any doubts that I had. [ ]

I also understand that my child's participation in this study is entirely voluntary and that I am free to withdraw permission to participate in this study at any time without affecting my child's usual treatment or his/her legal rights [ ]

I also understand that during the study, I will have to pay for blood investigations like Complete blood count, ESR and CRP. [ ]

I understand that this study involves taking by child's blood samples by venipuncture method [ ]

I understand that the study staff and institutional ethics committee members will not need my permission to look at my child's health records even if I withdraw from the trial. I agree to this access [ ]

I understand that my child's identity will not be revealed in any information released to third parties or published [ ]

I voluntarily agree and give consent for my child to take part in this study [ ]

Name of parent/guardian: Investigator's Name:
Signature: Investigator's Signature:
Date:

Name of witness:
Relation to participant:
Date:

**Christian Medical College, Vellore**

**Department of Pediatrics**

# CHILD ASSENT FORM

**Study Title: Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis**

**Study No:**

**Name:**

**Age:**

**Date Of Birth:**

- I understand that this study involves estimation of serum Calprotectin levels for assessment of disease activity in children with Juvenile Idiopathic Arthritis.
- I understand that if I am enrolled in this study, it involves collection of my blood samples by venipuncture method.
- I understand that my participation in the study is voluntary and that I amfree to withdraw from the study at any time, without giving any reason and without my medical care being affected.
- I understand that the study team, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records for the current study. I agree to this access. However, I understand that my identity will not be revealed in any information released to third parties or published.
- I have been explained about the study in detailand have had the opportunity to ask questions
- I agree to take part in the above study

**Your Name:** **Investigator's Name:**

**Your Signature:** **Investigators Signature:**

**Date:**

**Parents Signature / Thumb Impression**

# ChristianMedicalCollege, Vellore
# Department of Pediatrics

**Study Title: Serum Calprotectin Levels as a marker of disease activity in children with Juvenile Idiopathic Arthritis**

## Study Proforma

Study No: Date :

Name:

Age

Sex:

Hospital No:

Disease duration:

**Diagnosis:** JIA Types: Oligoarticular/Polyarticular RF +/Polyarticular RF -/Enthesitis related arthritis/psoriatic arthritis/systemic onset JIA

**Disease activity:** Active disease/Remission on medications/Remission off medications

**Laboratory Values:**

Hemoglobin:

Total Count:

Differential Count:

Platelet count:

ESR:

CRP:

Serum Calprotectin Level:

| slno | date | age | sex | diseasedur | diagnosis | diseaseact | hb | tc |
|---|---|---|---|---|---|---|---|---|
| 1 | 03/08/2016 | 6 | 1 | 6 | 3 | 1 | 8.6 | 9500 |
| 2 | 02/08/2016 | 9 | 1 | 1 | 4 | 1 | 8.6 | 23500 |
| 3 | 19/08/2016 | 16 | 1 | 13 | 7 | 1 | 8.6 | 9200 |
| 4 | 19/08/2016 | 14 | 1 | 3 | 7 | 1 | 9 | 8000 |
| 5 | 18/08/2016 | 14 | 2 | 6 | 3 | 1 | 9.9 | 8500 |
| 6 | 17/08/2016 | 6 | 2 | 2 | 7 | 1 | 7.1 | 12200 |
| 7 | 02/09/2016 | 3 | 2 | 2 | 7 | 1 | 9 | 10100 |
| 8 | 23/09/2017 | 12 | 2 | 1 | 3 | 1 | 11.2 | 8700 |
| 9 | 22/09/2016 | 16 | 1 | 6 | 7 | 1 | 11 | 9100 |
| 10 | 23/09/2017 | 10 | 2 | 6 | 7 | 1 | 6.9 | 11400 |
| 11 | 17/10/2016 | 6 | 2 | 2 | 1 | 1 | 10.8 | 9600 |
| 12 | 21/10/2016 | 8 | 2 | 3 | 7 | 1 | 8.8 | 10800 |
| 13 | 21/10/2016 | 12 | 2 | 4 | 7 | 1 | 8.1 | 19600 |
| 14 | 11/11/2016 | 14 | 1 | 3 | 7 | 1 | 11 | 37700 |
| 15 | 11/11/2016 | 14 | 2 | 7 | 7 | 1 | 9.2 | 20500 |
| 16 | 11/11/2016 | 6 | 1 | 1 | 7 | 1 | 10.9 | 14900 |
| 17 | 11/11/2016 | 14 | 2 | 1 | 3 | 1 | 10.2 | 9200 |
| 18 | 12/11/2016 | 13 | 2 | 1 | 7 | 1 | 9.2 | 28200 |
| 19 | 17/11/2016 | 5 | 1 | 1 | 7 | 1 | 10.4 | 31300 |
| 20 | 28/11/2016 | 15 | 1 | 6 | 3 | 1 | 10.9 | 10000 |
| 21 | 30/11/2016 | 13 | 1 | 5 | 7 | 1 | 9.3 | 6300 |
| 22 | 30/11/2016 | 16 | 1 | 7 | 7 | 1 | 9.7 | 17200 |
| 23 | 02/12/2016 | 14 | 1 | 3 | 2 | 1 | 10.6 | 11300 |
| 24 | 11/12/2016 | 16 | 1 | 6 | 4 | 1 | 10.9 | 12100 |
| 25 | 30/11/2016 | 11 | 1 | 2 | 5 | 1 | 11.5 | 18600 |
| 26 | 01/12/2016 | 16 | 1 | 2 | 4 | 1 | 10.4 | 8000 |
| 27 | 01/12/2016 | 5 | 1 | 4 | 3 | 1 | 7.6 | 15400 |
| 28 | 08/12/2016 | 14 | 1 | 6 | 3 | 1 | 8.7 | 6900 |
| 29 | 03/08/2016 | 6 | 1 | 1 | 7 | 2 | 12.3 | 17000 |
| 30 | 03/08/2016 | 13 | 1 | 4 | 3 | 2 | 13.1 | 7000 |
| 31 | 05/08/2016 | 10 | 2 | 2 | 3 | 2 | 11.8 | 12700 |
| 32 | 17/08/2016 | 11 | 2 | 2 | 3 | 2 | 13.3 | 10100 |
| 33 | 23/08/2016 | 16 | 1 | 9 | 4 | 2 | 2 | 7100 |
| 34 | 02/09/2016 | 11 | 1 | 1 | 3 | 2 | 11.4 | 10800 |
| 35 | 02/09/2016 | 10 | 1 | 6 | 7 | 2 | 12.7 | 11600 |
| 36 | 22/09/2016 | 8 | 2 | 4 | 2 | 2 | 10.5 | 11300 |
| 37 | 20/09/2016 | 12 | 1 | 3 | 3 | 2 | 12.2 | 7400 |
| 38 | 11/11/2016 | 15 | 2 | 4 | 7 | 2 | 12.6 | 11800 |
| 39 | 11/11/2016 | 12 | 2 | 9 | 3 | 2 | 12.5 | 10500 |
| 40 | 12/11/2016 | 7 | 2 | 1 | 1 | 2 | 11.2 | 7800 |
| 41 | 10/11/2016 | 7 | 2 | 5 | 1 | 2 | 11.9 | 12400 |
| 42 | 18/11/2016 | 2 | 2 | 1 | 1 | 2 | 10.2 | 17200 |
| 43 | 17/11/2016 | 13 | 1 | 1 | 4 | 2 | 13.3 | 5700 |
| 44 | 18/11/2016 | 7 | 2 | 2 | 3 | 2 | 12.9 | 7100 |
| 45 | 22/11/2016 | 10 | 2 | 1 | 7 | 2 | 13.4 | 5200 |
| 46 | 23/11/2016 | 8 | 2 | 6 | 3 | 2 | 14 | 6900 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 47 | 23/11/2016 | 16 | 2 | 4 | 1 | 2 | 10.4 | 6100 |
| 48 | 01/12/2016 | 10 | 1 | 4 | 7 | 2 | 11.1 | 10100 |
| 49 | 02/12/2016 | 7 | 2 | 2 | 1 | 2 | 11.3 | 10200 |
| 50 | 02/12/2016 | 12 | 1 | 4 | 3 | 2 | 11.9 | 9300 |
| 51 | 02/12/2016 | 16 | 1 | 5 | 3 | 2 | 15.7 | 8300 |
| 52 | 02/12/2016 | 10 | 2 | 9 | 3 | 2 | 11.9 | 5600 |
| 53 | 08/12/2016 | 9 | 1 | 3 | 7 | 2 | 12 | 6800 |
| 54 | 21/12/2016 | 7 | 1 | 6 | 3 | 2 | 12 | 9000 |
| 55 | 08/12/2016 | 16 | 1 | 2 | 4 | 1 | 11.8 | 10700 |
| 56 | 24/01/2017 | 13 | 2 | 7 | 3 | 1 | 9.7 | 8800 |
| 57 | 24/01/2017 | 11 | 2 | 1 | 7 | 1 | 9.1 | 39400 |
| 58 | 16/01/2017 | 10 | 2 | 7 | 7 | 1 | 10.7 | 11800 |
| 59 | 16/01/2017 | 3 | 2 | 1 | 7 | 1 | 6.9 | 24300 |
| 60 | 21/01/2017 | 10 | 2 | 1 | 3 | 1 | 10.6 | 18200 |
| 61 | 20/01/2017 | 8 | 2 | 4 | 7 | 1 | 8.8 | 17000 |
| 62 | 27/01/2017 | 14 | 2 | 3 | 7 | 1 | 10.9 | 9700 |
| 63 | 06/01/2017 | 13 | 1 | 6 | 7 | 1 | 11.2 | 150000 |
| 64 | 06/01/2017 | 15 | 2 | 4 | 7 | 1 | 11.4 | 12100 |
| 65 | 06/01/2017 | 7 | 1 | 6 | 1 | 1 | 6.1 | 200000 |
| 66 | 05/01/2017 | 9 | 1 | 6 | 7 | 1 | 9.6 | 28800 |
| 67 | 02/02/2017 | 6 | 1 | 4 | 7 | 1 | 8.7 | 12000 |
| 68 | 01/02/2017 | 5 | 2 | 1 | 7 | 1 | 10.2 | 16500 |
| 69 | 08/02/2017 | 4 | 1 | 2 | 3 | 1 | 9.3 | 17800 |
| 70 | 08/02/2017 | 10 | 2 | 3 | 1 | 1 | 12.4 | 11500 |
| 71 | 10/02/2017 | 8 | 2 | 1 | 3 | 1 | 10.1 | 9700 |
| 72 | 10/02/2017 | 13 | 1 | 4 | 4 | 1 | 11.7 | 9000 |
| 73 | 02/03/2017 | 14 | 2 | 2 | 7 | 1 | 10.5 | 16500 |
| 74 | 21/04/2017 | 16 | 2 | 5 | 7 | 1 | 11.4 | 10900 |
| 75 | 23/06/2017 | 15 | 1 | 1 | 3 | 1 | 11.8 | 14600 |
| 76 | 21/04/2017 | 16 | 1 | 7 | 5 | 1 | 12.3 | 8300 |
| 77 | 20/04/2017 | 15 | 1 | 1 | 3 | 1 | 11.9 | 10300 |
| 78 | 19/04/2017 | 15 | 1 | 1 | 7 | 1 | 9.4 | 18800 |
| 79 | 02/03/2017 | 7 | 1 | 2 | 7 | 1 | 10.4 | 12500 |
| 80 | 03/03/2017 | 16 | 1 | 14 | 7 | 1 | 12.1 | 7300 |
| 81 | 16/03/2017 | 13 | 1 | 7 | 7 | 1 | 13.3 | 23700 |
| 82 | 23/03/2017 | 12 | 1 | 1 | 4 | 1 | 10.8 | 9000 |
| 83 | 23/03/2017 | 14 | 1 | 3 | 4 | 1 | 10.5 | 8900 |
| 84 | 24/03/2017 | 14 | 1 | 9 | 7 | 1 | 8.6 | 27400 |
| 85 | 21/03/2017 | 12 | 1 | 5 | 7 | 1 | 11.4 | 18500 |
| 86 | 29/03/2017 | 10 | 2 | 7 | 7 | 1 | 10.2 | 12400 |
| 87 | 28/03/2017 | 13 | 1 | 5 | 7 | 1 | 9.1 | 6700 |
| 88 | 16/12/2016 | 6 | 1 | 2 | 7 | 1 | 9.2 | 20300 |
| 89 | 08/12/2016 | 14 | 1 | 1 | 3 | 2 | 11.2 | 6000 |
| 90 | 28/12/2016 | 11 | 1 | 4 | 7 | 1 | 11.1 | 15100 |
| 91 | 28/12/2016 | 16 | 2 | 5 | 2 | 2 | 13.4 | 7200 |
| 92 | 24/01/2017 | 3 | 2 | 1 | 7 | 2 | 12.1 | 14600 |
| 93 | 27/01/2017 | 6 | 1 | 1 | 7 | 2 | 10.4 | 13100 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 94 | 27/01/2017 | 10 | 2 | 1 | 7 | 2 | 11.2 | 14300 |
| 95 | 27/01/2017 | 13 | 1 | 1 | 5 | 2 | 13.1 | 6900 |
| 96 | 27/01/2017 | 12 | 1 | 2 | 3 | 2 | 13.4 | 9600 |
| 97 | 27/01/2017 | 9 | 2 | 7 | 7 | 2 | 10.3 | 8700 |
| 98 | 05/01/2017 | 13 | 1 | 1 | 4 | 2 | 11 | 7100 |
| 99 | 06/01/2017 | 10 | 2 | 1 | 2 | 2 | 12.2 | 6600 |
| 100 | 06/01/2017 | 14 | 2 | 5 | 3 | 2 | 11.9 | 8400 |
| 101 | 06/01/2017 | 10 | 1 | 6 | 3 | 2 | 11.9 | 12200 |
| 102 | 05/01/2017 | 7 | 1 | 2 | 7 | 2 | 12.5 | 10900 |
| 103 | 11/01/2017 | 15 | 1 | 3 | 4 | 2 | 11.5 | 6200 |
| 104 | 03/02/2017 | 14 | 2 | 4 | 3 | 2 | 12.4 | 7600 |
| 105 | 02/02/2017 | 10 | 2 | 4 | 3 | 2 | 13.8 | 7300 |
| 106 | 30/01/2017 | 12 | 2 | 3 | 3 | 2 | 10.6 | 9500 |
| 107 | 10/02/2017 | 16 | 2 | 5 | 3 | 2 | 14.1 | 11000 |
| 108 | 10/02/2017 | 16 | 2 | 6 | 3 | 2 | 12.7 | 8000 |
| 109 | 21/04/2017 | 6 | 1 | 1 | 3 | 2 | 14.1 | 10300 |
| 110 | 21/04/2017 | 14 | 2 | 4 | 7 | 2 | 10.6 | 7000 |
| 111 | 21/04/2017 | 16 | 1 | 5 | 3 | 2 | 12.9 | 6800 |
| 112 | 21/04/2017 | 12 | 1 | 6 | 4 | 2 | 12.6 | 9900 |
| 113 | 20/04/2017 | 10 | 1 | 1 | 7 | 2 | 10.7 | 8400 |
| 114 | 17/03/2017 | 12 | 1 | 8 | 7 | 2 | 11.8 | 13500 |
| 115 | 23/03/2017 | 5 | 2 | 2 | 7 | 2 | 12.4 | 10400 |
| 116 | 21/03/2017 | 15 | 1 | 4 | 4 | 2 | 11.6 | 6700 |
| 117 | 22/03/2017 | 12 | 2 | 8 | 4 | 2 | 11.3 | 5500 |
| 118 | 11/04/2017 | 13 | 1 | 5 | 4 | 3 | 13.1 | 12200 |
| 119 | 12/04/2017 | 16 | 1 | 9 | 4 | 2 | 14.6 | 7100 |
| 120 | 23/06/2017 | 6 | 1 | 2 | 3 | 2 | 13.1 | 7100 |
| 121 | 22/06/2017 | 16 | 1 | 5 | 4 | 2 | 12.6 | 8000 |

| dcn | dcl | dce | platelet | esr | crp | serum |
|---|---|---|---|---|---|---|
| 69 | 16 | 0 | 609600 | 57 | 128 | 11970 |
| 65 | 23 | 0 | 684000 | 59 | 54 | 3146 |
| 57 | 34 | 1 | 398000 | 57 | 34 | 1420 |
| 56 | 34 | 2 | 665000 | 51 | 75 | 1000 |
| 83 | 13 | 1 | 410000 | 54 | 46 | 2228 |
| 57 | 32 | 1 | 728000 | 52 | 119 | 1703 |
| 38 | 54 | 1 | 533000 | 27 | 78 | 1693 |
| 48 | 45 | 1 | 547000 | 45 | 111 | 651 |
| 56 | 29 | 7 | 385000 | 43 | 16 | 1818 |
| 70 | 22 | 4 | 751000 | 59 | 170 | 2028 |
| 71 | 17 | 2 | 352000 | 60 | 61 | 1517 |
| 65 | 25 | 2 | 560000 | 56 | 36 | 2740 |
| 72 | 18 | 2 | 730000 | 79 | 163 | 1659 |
| 91 | 8 | 0 | 557000 | 41 | 201 | 1050 |
| 82 | 13 | 0 | 370000 | 59 | 136 | 1163 |
| 77 | 17 | 1 | 434000 | 61 | 190 | 1770 |
| 68 | 19 | 4 | 382000 | 48 | 20 | 1774 |
| 81 | 14 | 0 | 322000 | 9 | 161 | 1024 |
| 88 | 8 | 0 | 564000 | 54 | 119 | 2033 |
| 73 | 24 | 1 | 197000 | 52 | 148 | 1163 |
| 52 | 37 | 4 | 509000 | 52 | 61 | 7690 |
| 49 | 32 | 14 | 405000 | 38 | 15 | 769 |
| 62 | 28 | 1 | 594000 | 29 | 41 | 850 |
| 71 | 14 | 6 | 249000 | 43 | 169 | 2380 |
| 74 | 21 | 1 | 516000 | 44 | 44 | 2028 |
| 49 | 38 | 4 | 241000 | 60 | 45 | 1110 |
| 54 | 40 | 1 | 222000 | 90 | 77 | 6900 |
| 71 | 21 | 4 | 491000 | 67 | 107 | 3600 |
| 58 | 33 | 0 | 206000 | 6 | 3 | 1020 |
| 32 | 37 | 0 | 345000 | 5 | 3 | 680 |
| 50 | 37 | 3 | 419000 | 27 | 3 | 700 |
| 62 | 28 | 3 | 263000 | 37 | 3 | 860 |
| 32 | 43 | 14 | 340000 | 18 | 3 | 857 |
| 51 | 28 | 11 | 322000 | 9 | 3 | 780 |
| 81 | 12 | 0 | 221000 | 8 | 4 | 870 |
| 32 | 54 | 6 | 145000 | 16 | 3 | 525 |
| 55 | 35 | 4 | 178000 | 20 | 3 | 800 |
| 61 | 30 | 0 | 378000 | 3 | 3 | 980 |
| 59 | 34 | 1 | 495000 | 22 | 3 | 415 |
| 39 | 50 | 4 | 376000 | 17 | 3 | 240 |
| 55 | 38 | 2 | 360000 | 15 | 3 | 650 |
| 70 | 24 | 2 | 430000 | 28 | 3 | 953 |
| 53 | 35 | 1 | 212000 | 15 | 3 | 619 |
| 53 | 38 | 2 | 413000 | 20 | 3 | 818 |
| 38 | 47 | 8 | 254000 | 17 | 3 | 666 |
| 52 | 42 | 2 | 350000 | 4 | 3 | 663 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 58 | 34 | 0 | 248000 | 14 | 3 | 450 |
| 76 | 17 | 2 | 409000 | 5 | 3 | 810 |
| 50 | 36 | 8 | 606000 | 13 | 3 | 372 |
| 42 | 45 | 6 | 446000 | 10 | 3 | 378 |
| 55 | 31 | 3 | 217000 | 3 | 3 | 741 |
| 51 | 28 | 12 | 188000 | 25 | 3 | 16 |
| 38 | 20 | 7 | 191000 | 9 | 3 | 124 |
| 54 | 34 | 2 | 360000 | 10 | 3 | 599 |
| 75 | 18 | 1 | 306000 | 25 | 176 | 3006 |
| 57 | 37 | 1 | 480000 | 55 | 52 | 5762 |
| 82 | 6 | 0 | 370000 | 67 | 110 | 4928 |
| 80 | 15 | 0 | 573000 | 62 | 169 | 7138 |
| 79 | 18 | 0 | 380000 | 2 | 181 | 2398 |
| 81 | 13 | 0 | 610000 | 37 | 395 | 6534 |
| 57 | 36 | 2 | 670000 | 67 | 120 | 4792 |
| 51 | 41 | 2 | 390000 | 32 | 180 | 10528 |
| 88 | 6 | 6 | 540000 | 40 | 47 | 8864 |
| 73 | 19 | 1 | 468000 | 20 | 48 | 10904 |
| 56 | 36 | 0 | 106000 | 22 | 19 | 4608 |
| 85 | 9 | 1 | 310000 | 9 | 201 | 4014 |
| 81 | 14 | 1 | 340000 | 51 | 54 | 5598 |
| 80 | 15 | 0 | 286000 | 57 | 201 | 2366 |
| 51 | 36 | 7 | 640000 | 34 | 36 | 2201 |
| 75 | 17 | 0 | 510000 | 56 | 58 | 3960 |
| 50 | 36 | 2 | 392000 | 26 | 51 | 4048 |
| 58 | 34 | 2 | 370000 | 34 | 12 | 3797 |
| 78 | 15 | 0 | 429000 | 55 | 111 | 2045 |
| 74 | 21 | 0 | 590000 | 30 | 73 | 10900 |
| 80 | 12 | 2 | 403000 | 33 | 59 | 2513 |
| 75 | 17 | 1 | 240000 | 22 | 40 | 2255 |
| 67 | 22 | 2 | 280000 | 45 | 11 | 2780 |
| 49 | 36 | 5 | 711000 | 55 | 55 | 850 |
| 69 | 23 | 2 | 528000 | 53 | 201 | 3667 |
| 54 | 38 | 0 | 334000 | 26 | 80 | 14310 |
| 92 | 5 | 0 | 515000 | 35 | 57 | 3930 |
| 76 | 15 | 0 | 421000 | 32 | 92 | 2466 |
| 57 | 18 | 18 | 279000 | 53 | 38 | 2460 |
| 92 | 6 | 0 | 505000 | 55 | 101 | 5650 |
| 86 | 11 | 1 | 409000 | 58 | 201 | 4698 |
| 64 | 28 | 1 | 613000 | 57 | 127 | 11150 |
| 45 | 40 | 6 | 370000 | 71 | 76 | 3570 |
| 80 | 15 | 2 | 392000 | 67 | 170 | 2274 |
| 50 | 41 | 3 | 263000 | 38 | 3 | 2820 |
| 83 | 12 | 1 | 425000 | 54 | 114 | 9240 |
| 62 | 28 | 3 | 335000 | 17 | 3 | 388 |
| 49 | 42 | 3 | 522000 | 10 | 3 | 2660 |
| 59 | 32 | 3 | 690000 | 9 | 3 | 3600 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 78 | 16 | 0 | 460000 | 20 | 3 | 456 |
| 52 | 35 | 2 | 280000 | 8 | 3 | 3180 |
| 58 | 30 | 5 | 330000 | 17 | 4 | 7240 |
| 39 | 43 | 7 | 480000 | 14 | 3 | 712 |
| 28 | 50 | 14 | 284000 | 20 | 3 | 2340 |
| 55 | 33 | 3 | 374000 | 17 | 3 | 2140 |
| 58 | 30 | 4 | 274000 | 21 | 3 | 2400 |
| 43 | 33 | 19 | 386000 | 6 | 3 | 1420 |
| 52 | 39 | 3 | 260000 | 20 | 4 | 1750 |
| 61 | 30 | 2 | 279000 | 8 | 3 | 4160 |
| 50 | 35 | 4 | 306000 | 9 | 3 | 5620 |
| 49 | 40 | 3 | 287000 | 5 | 3 | 3660 |
| 44 | 42 | 9 | 176000 | 32 | 3 | 728 |
| 66 | 24 | 5 | 240000 | 9 | 3 | 2560 |
| 54 | 42 | 0 | 220000 | 9 | 3 | 622 |
| 34 | 53 | 6 | 375000 | 5 | 3 | 3600 |
| 45 | 45 | 2 | 370000 | 13 | 3 | 3120 |
| 60 | 30 | 4 | 270000 | 14 | 3 | 5700 |
| 47 | 40 | 7 | 409000 | 20 | 3 | 2660 |
| 58 | 28 | 7 | 358000 | 8 | 3 | 1060 |
| 70 | 22 | 1 | 465000 | 20 | 3 | 2920 |
| 52 | 35 | 7 | 405000 | 10 | 3 | 7780 |
| 46 | 40 | 4 | 270000 | 20 | 3 | 2760 |
| 40 | 54 | 1 | 218000 | 10 | 3 | 1360 |
| 34 | 28 | 30 | 84000 | 19 | 3 | 5620 |
| 38 | 42 | 10 | 281000 | 8 | 3 | 3200 |
| 23 | 59 | 7 | 328000 | 5 | 3 | 2080 |
| 42 | 43 | 5 | 143000 | 9 | 4 | 3220 |